

Thrombin Peptide TP508 (Chrysalin®) in Tissue Repair and Endothelial Function

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Galveston, TX

*Medical Countermeasures for
Radiation Combined Injury
Washington D.C.*

March 27, 2007



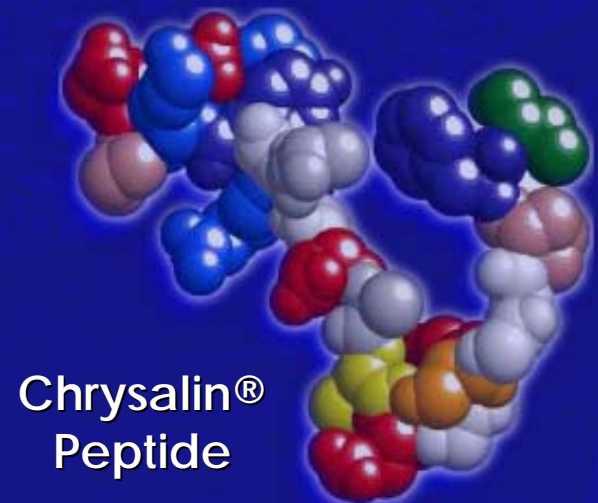
Chrysalis BioTechnology, Inc.

Founded in 1995 to develop novel thrombin peptides for tissue repair (SBIR, NIAID)

Spin-out of the University of Texas Medical Branch, Galveston, TX

Lead product TP508 (Chrysalin®)

Acquired by OrthoLogic Corp (OLGC) August, 2004.



Disclosure

- As the founder of Chrysalis, Dr. Carney has a financial interest in this technology and is a consultant for OrthoLogic.
- Conflicts of interest are managed by the University of Texas Medical Branch (UTMB) Conflict of Interest Committee.



Outline:

- Thrombin and TP508's role in cellular activation
- Preclinical studies and clinical trials with TP508 - most effective in chronic wounds or “at risk” patients
- Studies suggesting a universal signaling mechanism
- Ability of TP508 to reverse Endothelial Dysfunction

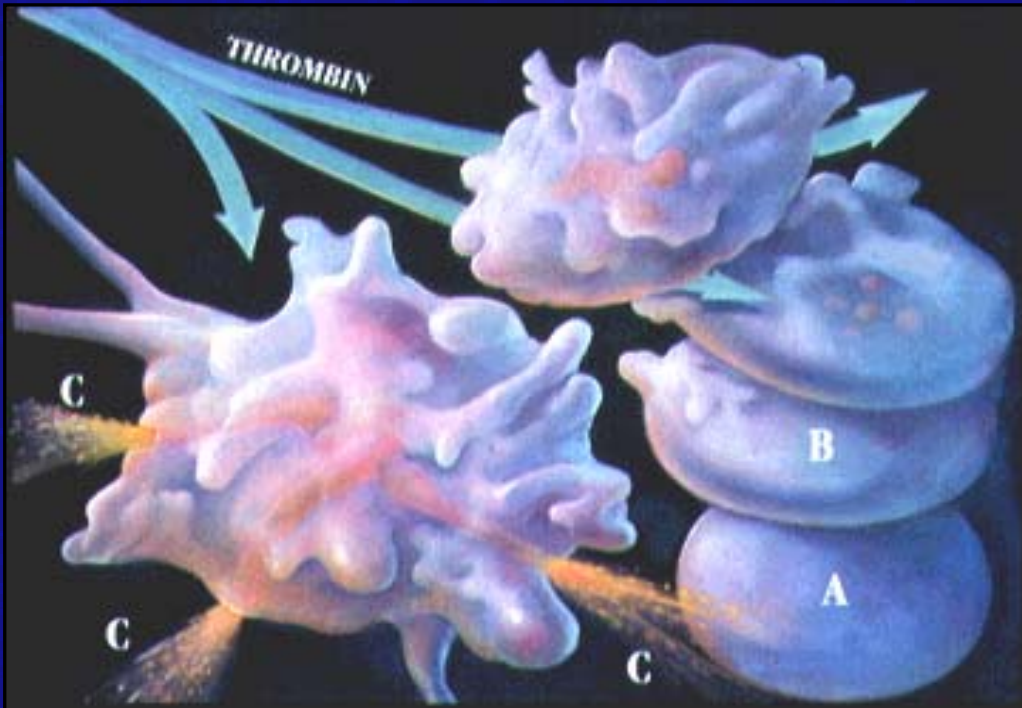
Key roles of thrombin in tissue repair

Homeostatic Effects

- Cleaves fibrinogen to form fibrin clots
- Activates blood platelets
- Mediated exclusively by proteolytically active thrombin

Inflammatory and Proliferative

- Stimulates cell migration, proliferation, matrix deposition, cytokine production, and differentiation
- Mediated by proteolytically active and inactive thrombin fragments



Early and Late effects of thrombin

■ Early

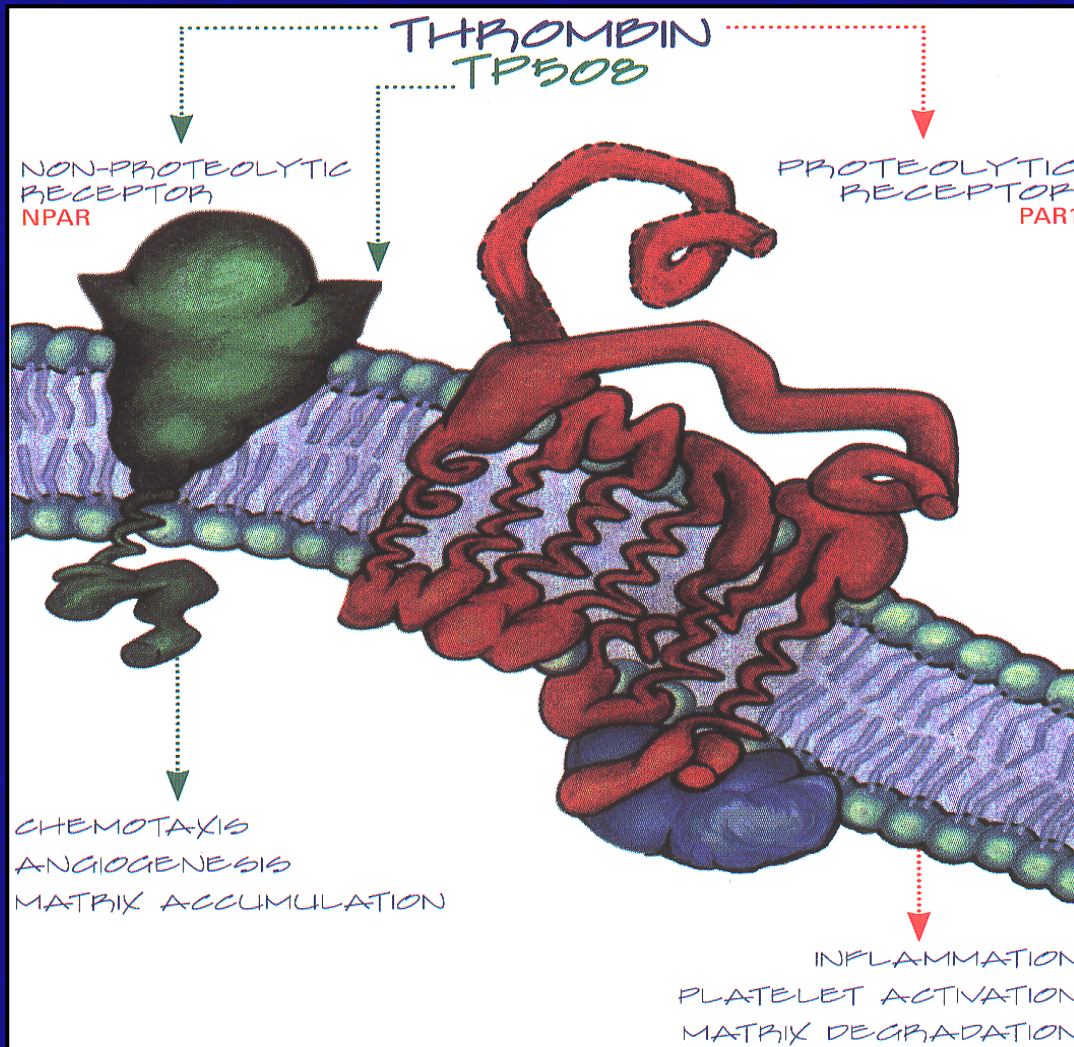
- Thrombin released at site of injury activates PAR receptors to produce pro-inflammatory responses
- Thrombin trapped in fibrin clot

■ Late

- Degradation releases peptide fragments of thrombin
- Peptides stimulate recruitment and activation of inflammatory cells, endothelial cells, fibroblasts and progenitor cells by non-proteolytic mechanism
- Peptides also attenuate inflammatory response and promote later stages of healing

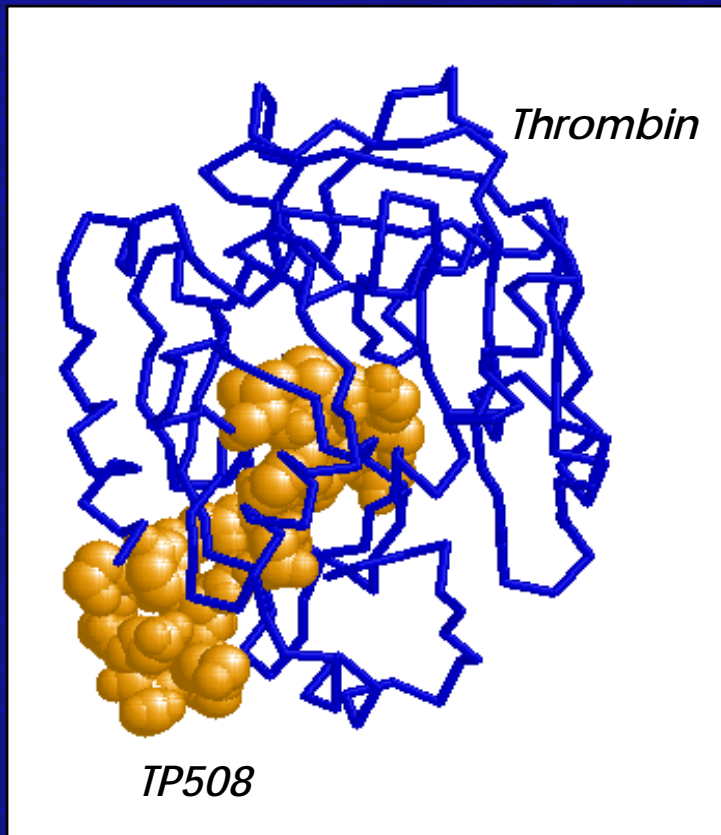
TP508 is a synthetic thrombin peptide

Receptors for thrombin and TP508 peptide



- Multiple thrombin receptors on cells
- Proteolytically activated receptors (PAR1-4)
- Non-Proteolytically activated receptor (NPAR)
- TP508 has no proteolytic activity and acts through NPAR

Thrombin Peptide TP508 (Chrysalin[®])

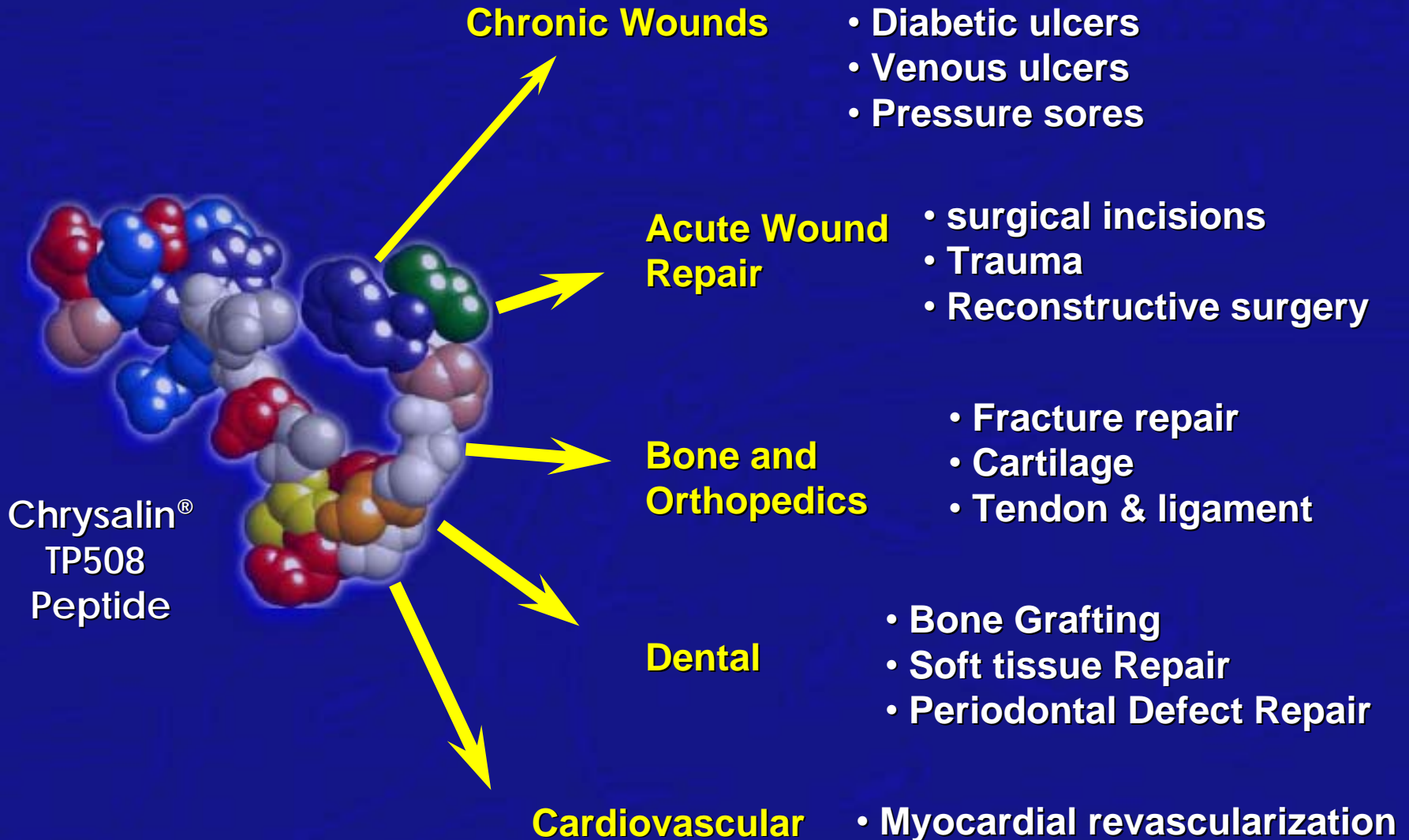


- 23 amino acid peptide representing the receptor binding domain sequence of human thrombin.
- Mimics many of thrombin's effects on cells but has no proteolytic activity
- Initiates repair of multiple tissues
- Excellent safety profile
- Synthetically manufactured at low cost
- Currently being tested for clinical applications

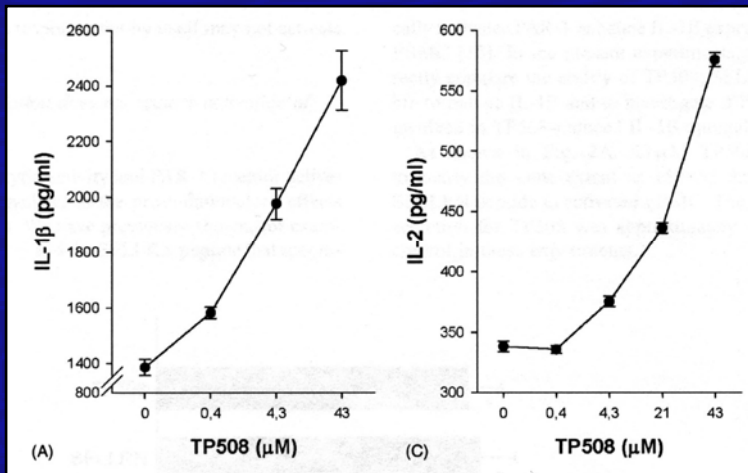
Target Cell Populations

- Inflammatory Cells
 - Fibroblasts
 - Endothelial Cells
 - Epithelial Cells (Keratinocytes)
 - Osteoblasts
 - Chondrocytes
 - Stem Cells
-
- All of these cells have high-affinity binding sites for thrombin and TP508
 - Effects include: chemotaxis, proliferation, angiogenesis, differentiation, prevention of, or protection from, apoptosis
 - Gene arrays show dramatic effects suggesting that peptide initiates a major signaling cascade within cells

Chrysalin Tissue Repair Applications

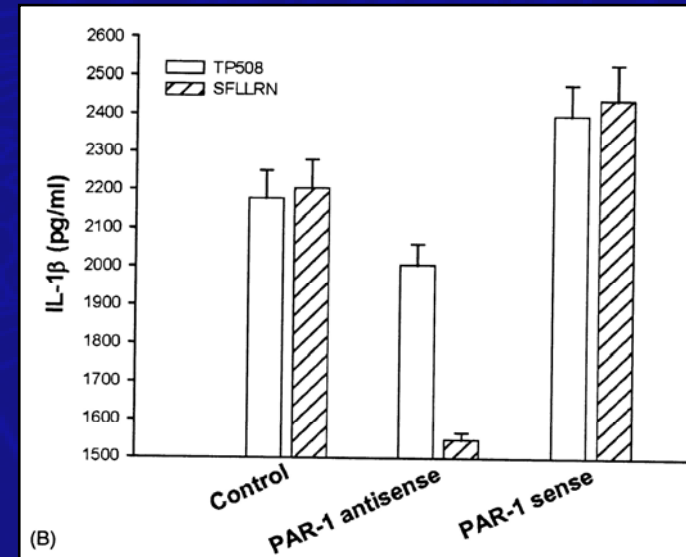


TP508 initiates cytokine production in monocytes



- PHA activated PBMCs treated with TP508 for 18 hours

Naldini et. al. Peptides; 25:1917-1926 (2004)

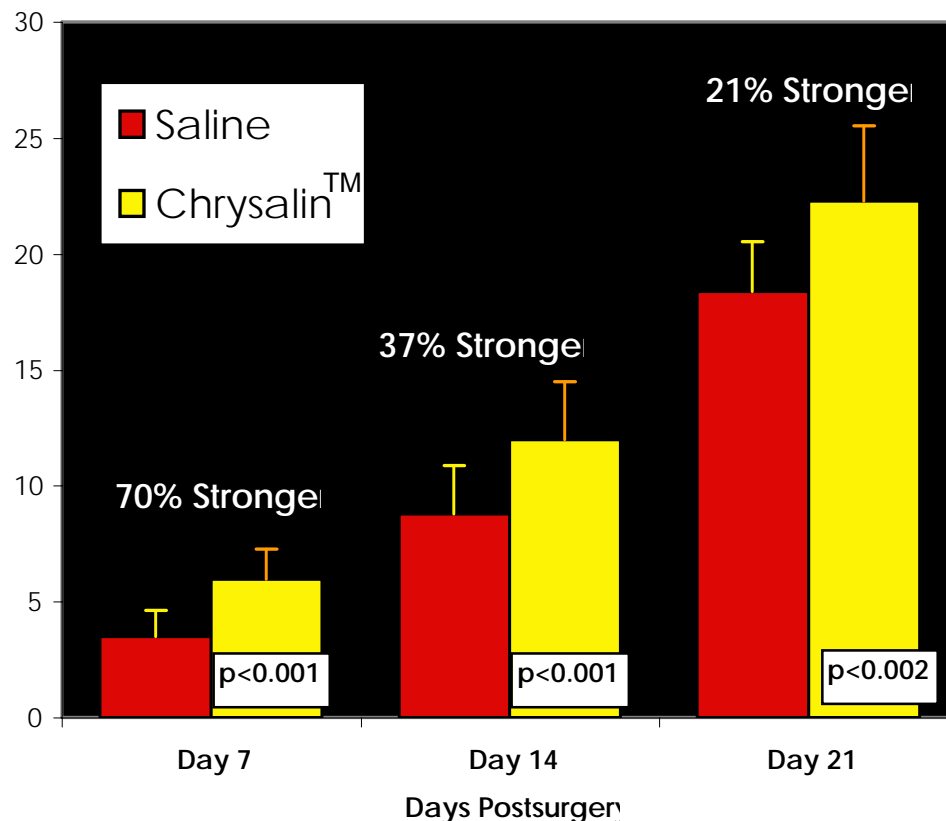


- TP508 stimulates IL-1 production to same level as SFLLRN
- PAR1 antisense oligos inhibit stimulation by SFLLRN, but not by TP508
- TP508 does not act through PAR1

Preclinical Studies

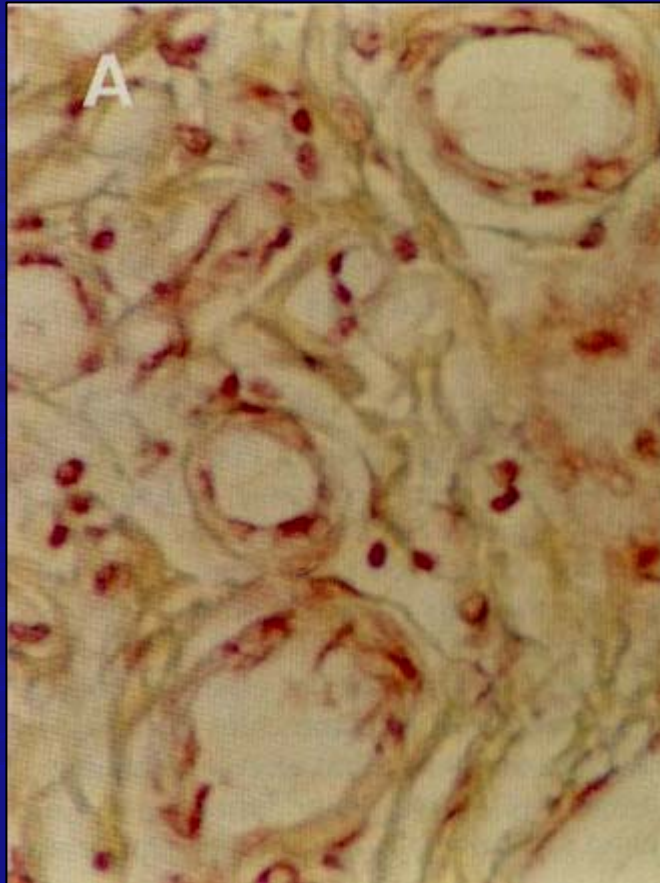
- Dermal wounds
- Fracture repair
- Segmental bone defects
- Periodontal bone filling
- Cartilage repair
- Myocardial revascularization
- Endothelial dysfunction

Chrysalin Stimulates Incisional Wound Healing--Breaking Strength in Rats

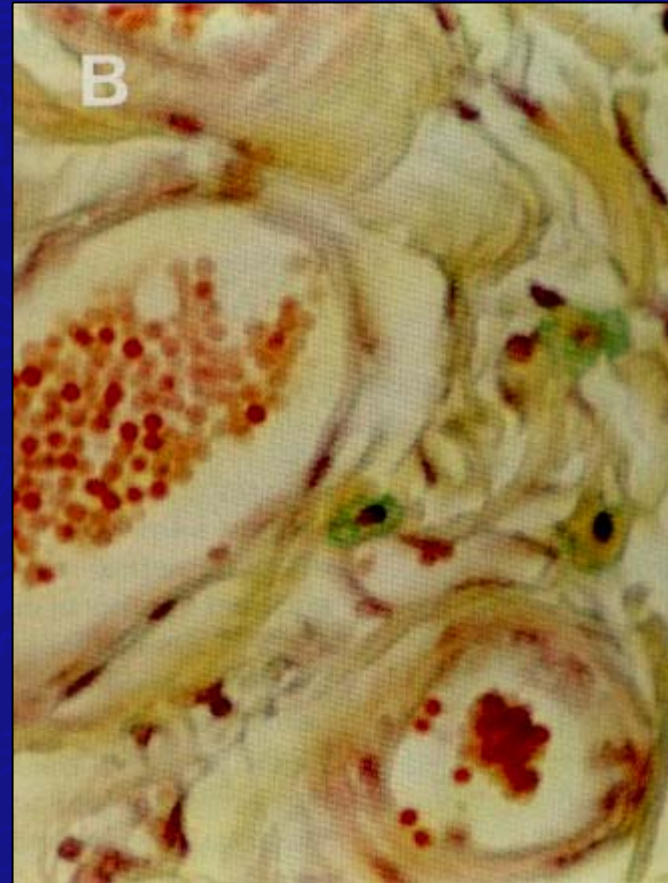


- Single topical application of Chrysalin
- 70% increase in breaking strength day 7
- Controls day 11 equal to treated day 7
- ~4 day acceleration of healing
- Increased day 7 revascularization and collagen formation

Chrysalin Effect on Blood Vessel Formation and Maturation -- Day 7

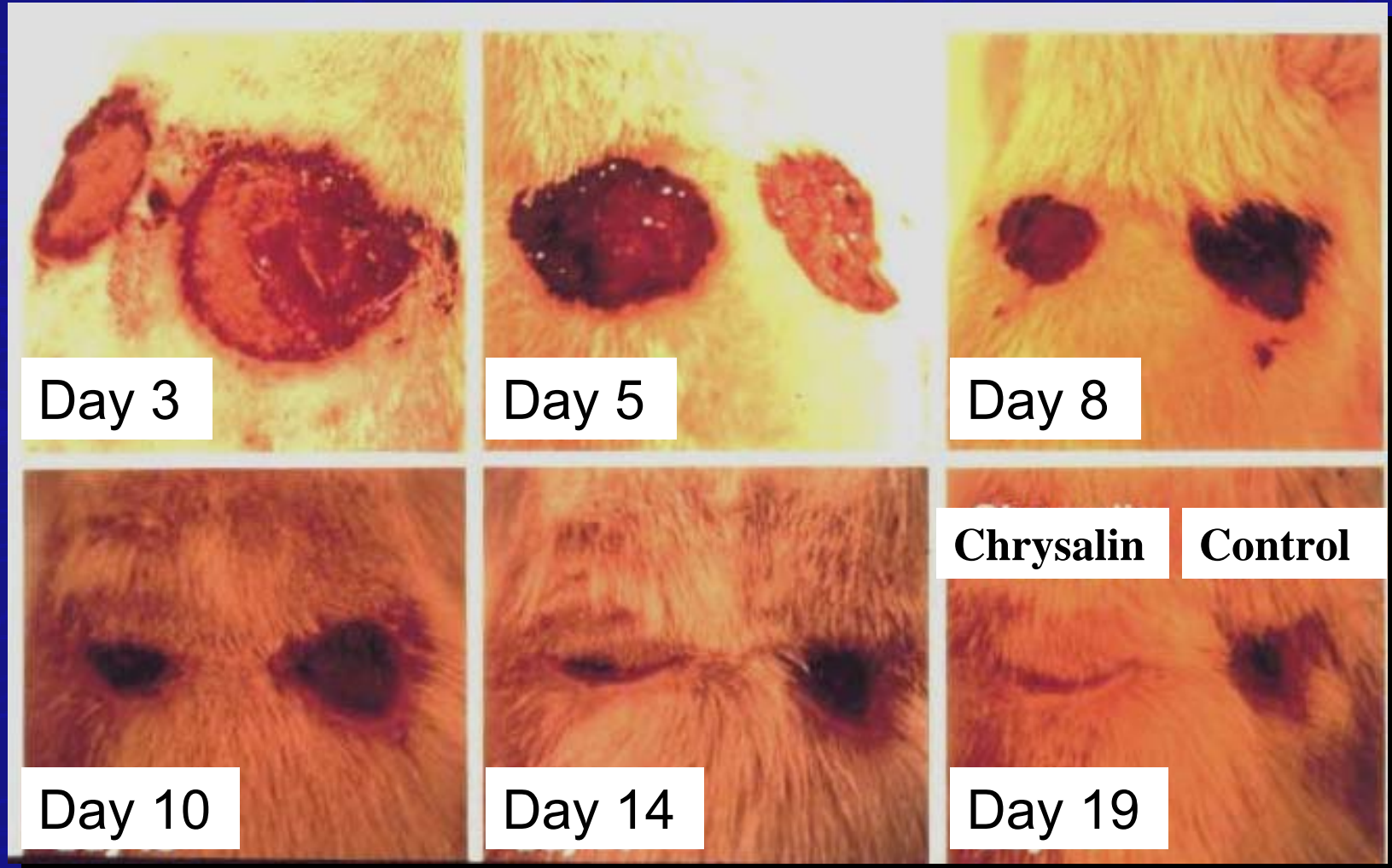


Control

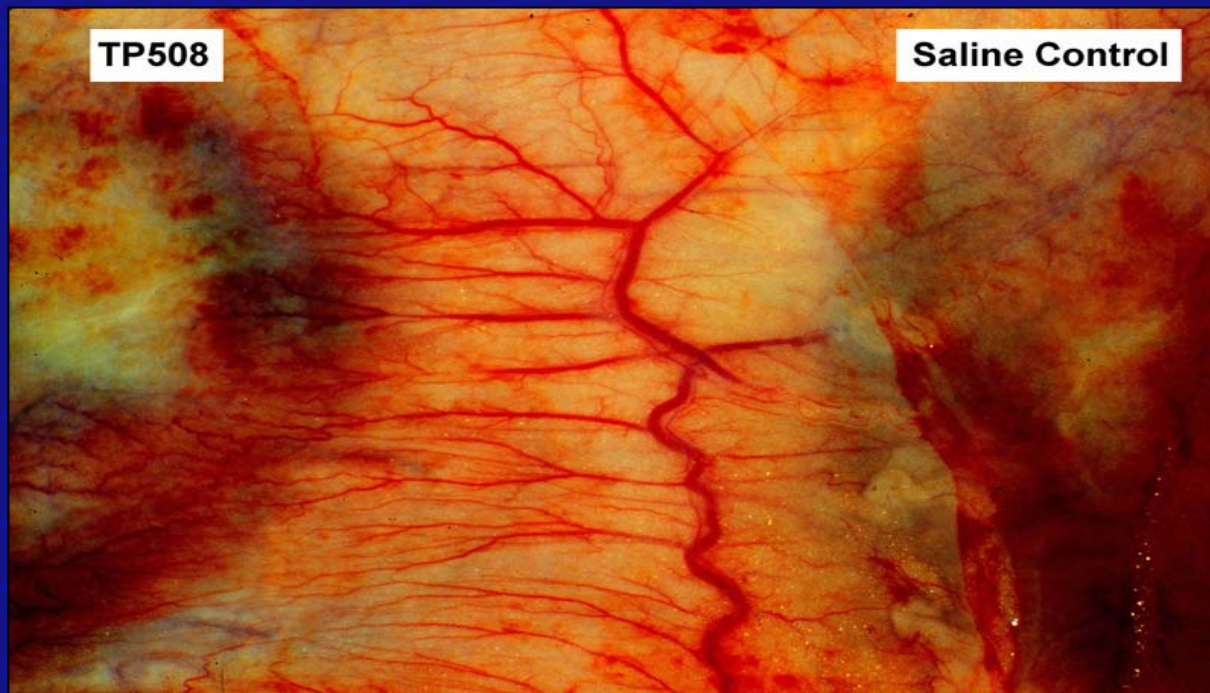
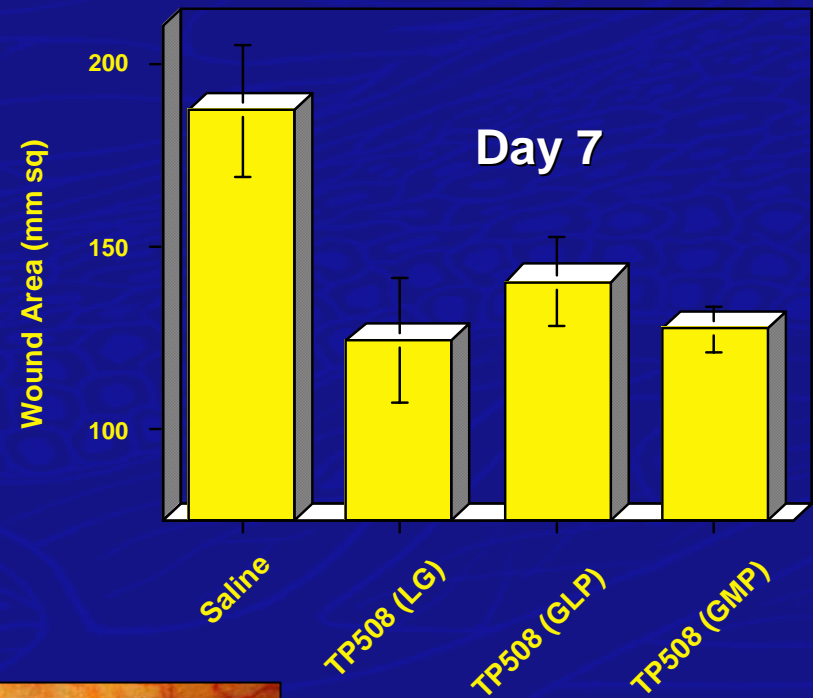


Chrysalin

Single topical application of Chrysalin to rat 2cm dermal wounds



*TP508 Stimulates
Full-dermal
Wound Closure
...and
Revascularization*

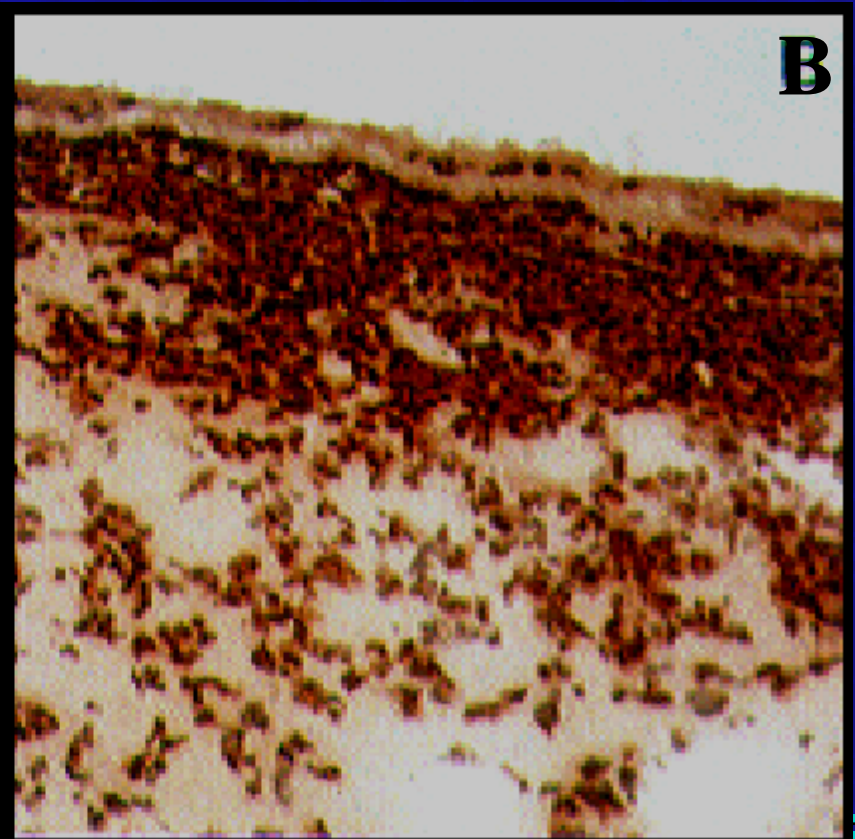
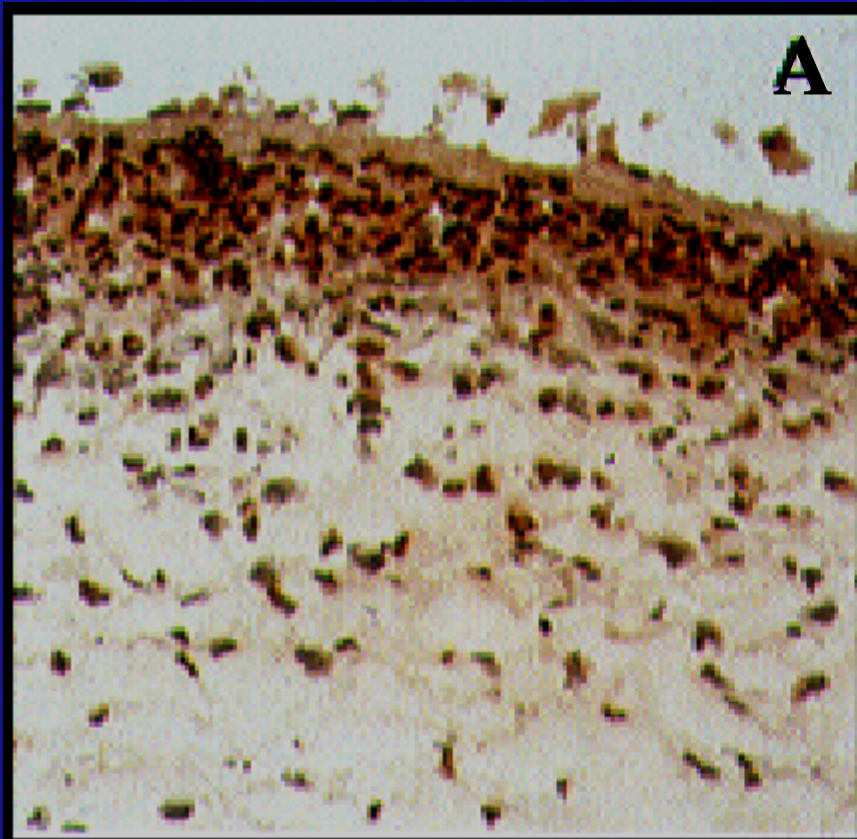


Wound Repair and
Regeneration 8: 204-
215 (2000)

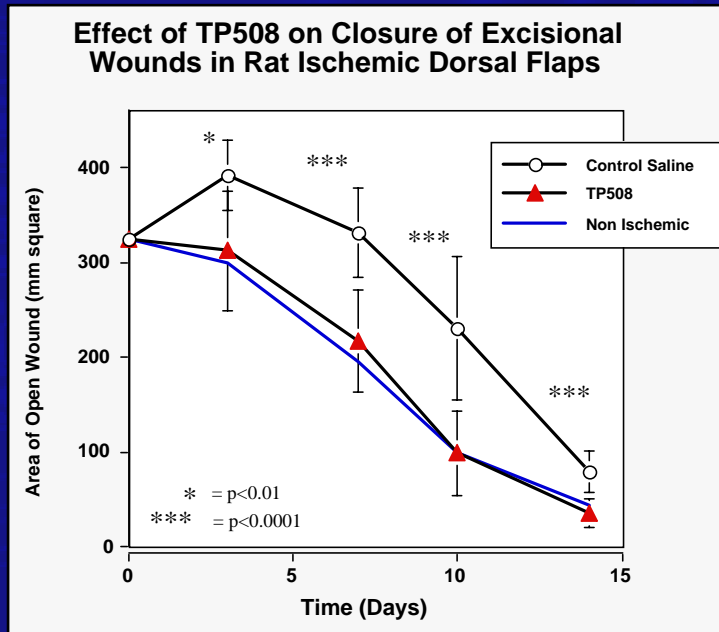
TP508 Stimulates PMN Recruitment and Activation - 24 hr

Saline

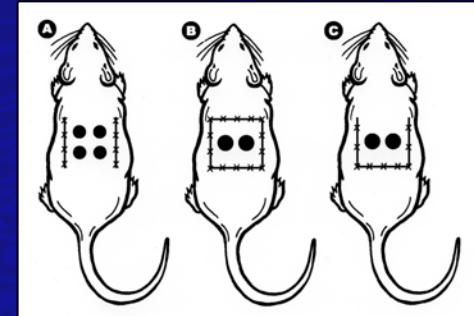
TP508



Does TP508 work in impaired wound healing models?



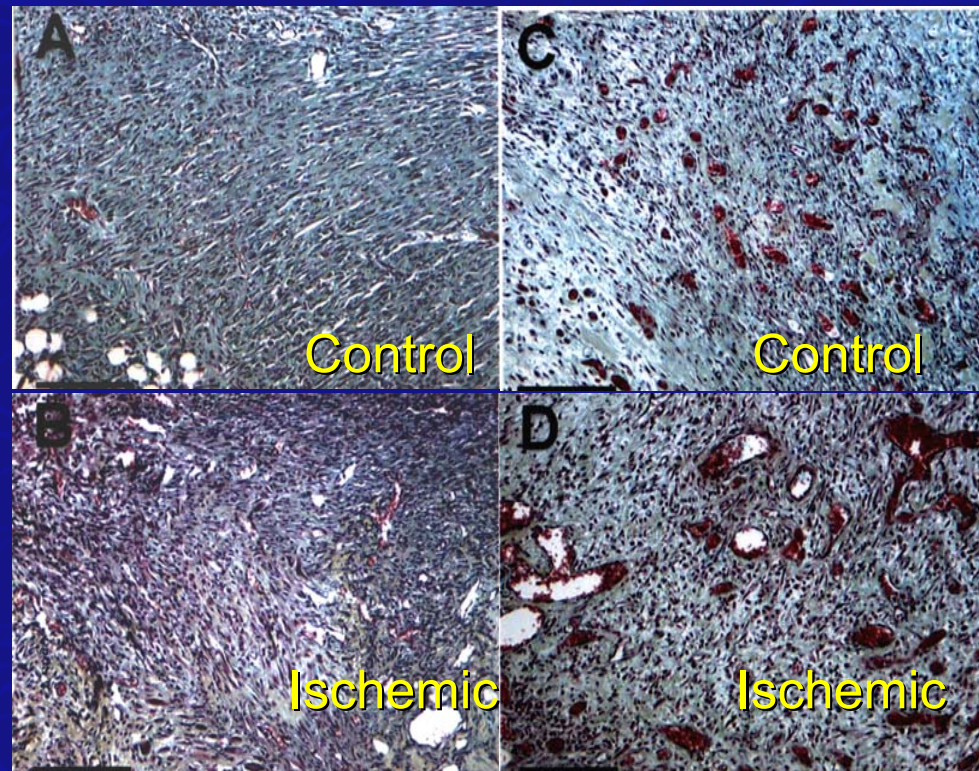
Wound Repair and
Regeneration 8: 517-529 (2000).



surgically-induced ischemia

Saline

Chrysalin



Early evaluation of TP517 effects on radiation impaired rat models - T. Mustoe

Model 1 Total Body Irradiation - 800 cGy (cobalt 60)

- monocytopenia (5% of normal)
- Bone marrow depression
- Platelet count <10% of normal
- spares skin and fibroblasts
- 43% reduction in wound breaking strength

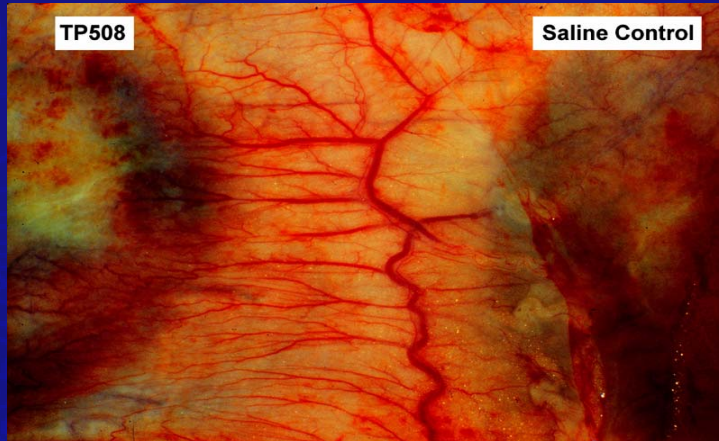
Model 2 Surface Irradiation - (Megavoltage electron beam therapy) Surface 2500 cGy, <300 at 10 mm deep and < 30cGy at 1 cm

- normal circulating monocytes
- No apparent effect on bone marrow
- Normal platelets and monocytes
- 56% reduction in wound breaking strength

TP517 effects different from PDGF and TGF β

- PDGF increases healing in surface irradiation but not whole body irradiation, suggesting need for recruitment of monocytes etc.
- TGF β increases healing after whole body irradiation, but not Surface irradiation consistent with direct effects on fibroblasts and skin cells
- TP517 ~40% increase in whole body and ~30% increase in surface irradiation. Thus, multiple effects on cell recruitment and activation of resident cells (fibroblasts, endothelial cells, etc.) to overcome effects of irradiation

Dermal Wound Healing



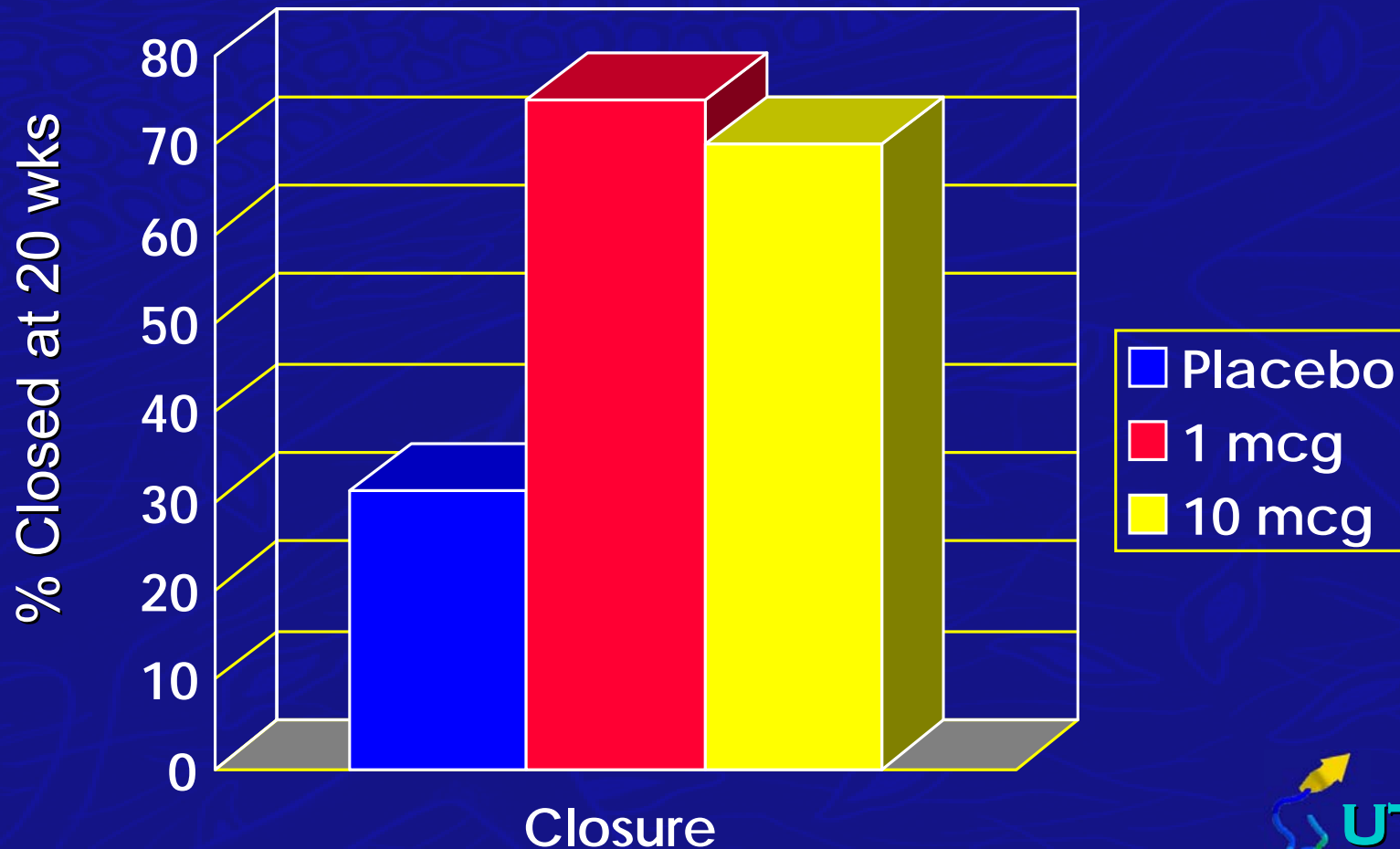
Pilot Phase 1,2 Human Clinical Trial Completed: Diabetic Foot Ulcers



- Double-blinded, placebo-controlled study - 60 patients - 4 centers
- Twice weekly topical application of saline, 1 μ g Chrysalin, or 10 μ g Chrysalin
- 20 week study
- Offloading and standard care for all patients
- Data published in Wound Repair and Regeneration 15: 23-34 (2007).

Chrysalin is an investigational drug not yet approved for commercial use

Chrysalin more than doubles the incidence of complete healing of Foot Ulcers (% Healed at 20 weeks)



Kaplan-Meier Analysis of Time to 100% Wound Closure

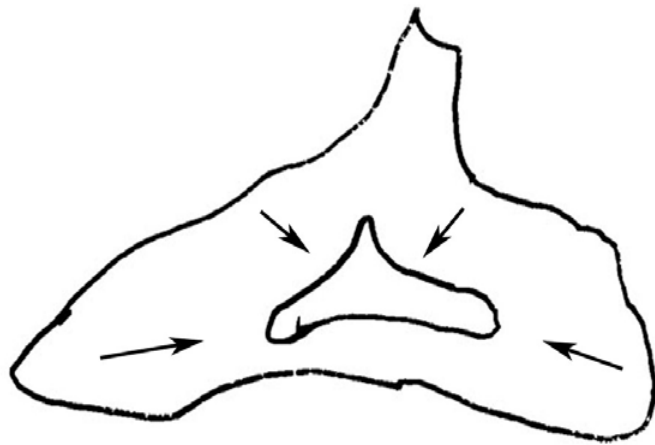
TREATMENT	TOTAL PATIENTS	TOTAL HEALED	TOTAL CENSORED	MEDIANTIME (DAYS)
Saline	13	4	9	Not reached
1 mcg	12	9	3	94
10 mcg	10	7	3	71.5*
1 or 10 mcg	22	16	6	94**

Log-Rank P-Values: Saline vs. 1 mcg: 0.1071

*Saline vs. 10 mcg: 0.0333

**Saline vs. 1 or 10 mcg: 0.0403

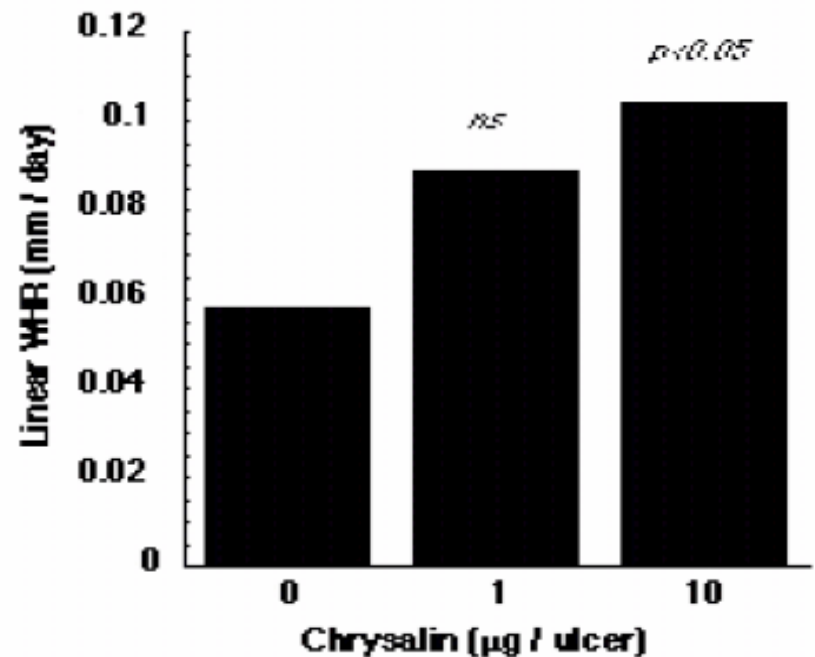
1 mcg vs. 10 mcg: 0.3440



$$\text{WHR} = [(\text{Area T0} - \text{Area TX}) / ((\text{Perimeter T0} + \text{Perimeter TX}) / 2)] / \text{days (TX)}$$

Chrysalin Effect on Linear Wound Healing

*Rate almost
double that of
placebos*



Case #2: 47 year-old male



Location: Heel

Post-Debridement Baseline Area:
13.1cm²

Treatment: 10 mcg Chrysalin

Healed in 102 days

Wound Repair and Regeneration 15: 23-34 (2007).

Ulcers located on the heel of the foot

Treatment	Total Patients	Mean Area (Day 0)	Total Healed	Percent Healed	Mean WHR (mm/day)
SALINE	5	5.51	0	0	0.024
1.0 µg Chrysalin	3	3.61	3	100	0.035
10 µg Chrysalin	4	6.19	3	75	0.063**
1.0 or 10.0 µg	7	5.39	6	85.7*	.051***

*Chi-square test: Saline vs. 1.0 or 10 µg $p = 0.021$

**Student T test: Saline vs. 10µg $p = 0.014$

*** Student T test: Saline vs. 1 or 10µg $p=0.06$

Wound Repair and Regeneration 15: 23-34 (2007).



Diabetic Ulcer Conclusions

- No drug-related adverse events
- Significant effects in foot ulcer sub-population
 - Increased 20-wk total percent closure (72%vs 33%, $p = 0.05$)
 - Cut median time to closure in half ($p = 0.033$)
 - Nearly doubled linear rate of wound closure (82%)
 - Increased probability of 60-day closure from 25% to over 60%
- Surprising potential efficacy with large heel ulcers



Orthopedic Applications

- ☐ Fracture Repair
- ☐ Segmental Defects
- ☐ Spine Fusion
- ☐ Cartilage Repair
- ☐ Ligament/Tendon

Preclinical foundations: Rat Fracture Healing

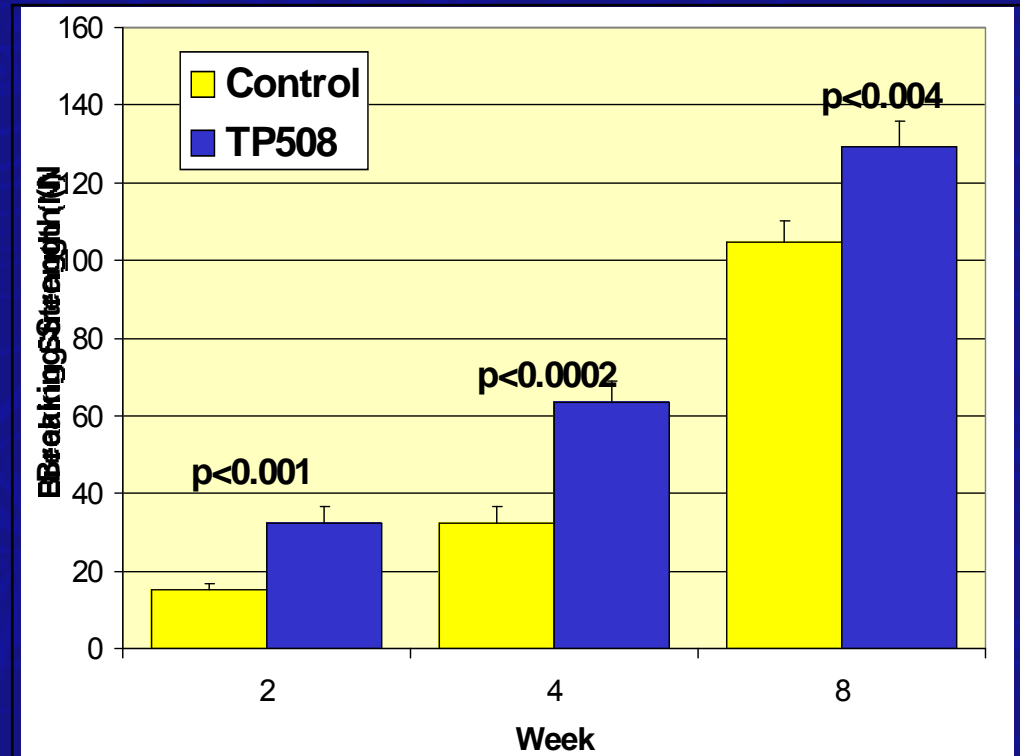
2 Weeks



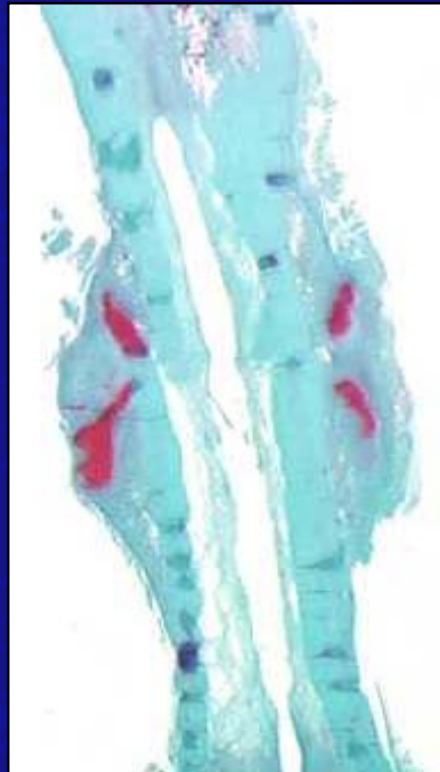
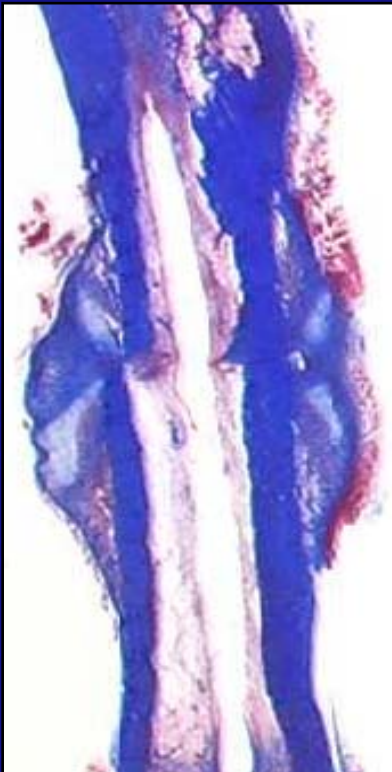
Cont



TP508



TP508 - Histological Analysis 3 weeks post-fracture

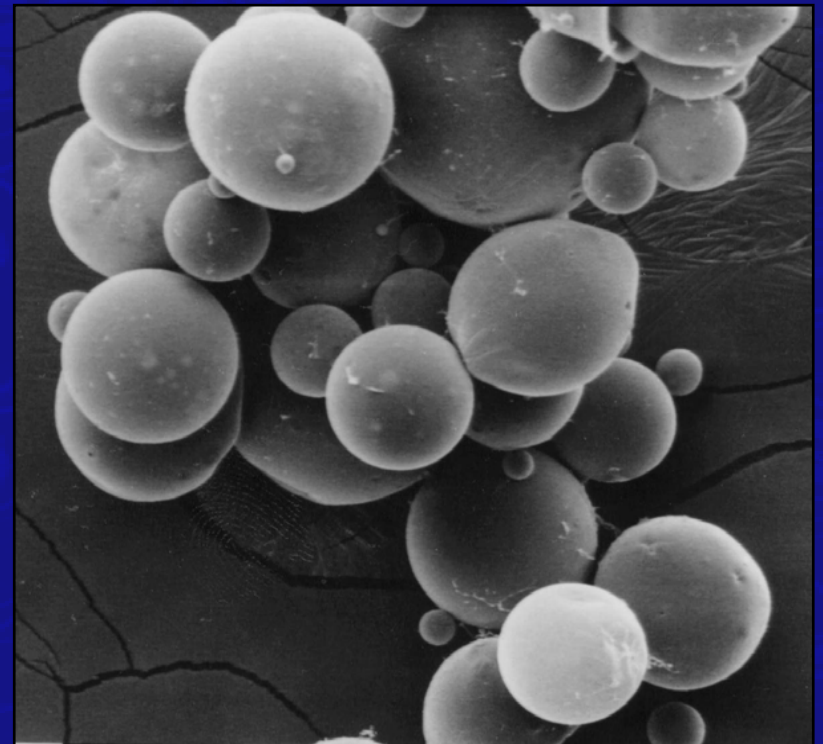


Control

TP508

PLGA Chrysalin Formulation

- Chrysalin incorporated in resorbable PLGA matrix
- Used in segmental defect and cartilage defect studies



TP508 Microspheres

Rabbit 1.5cm critical-size defects

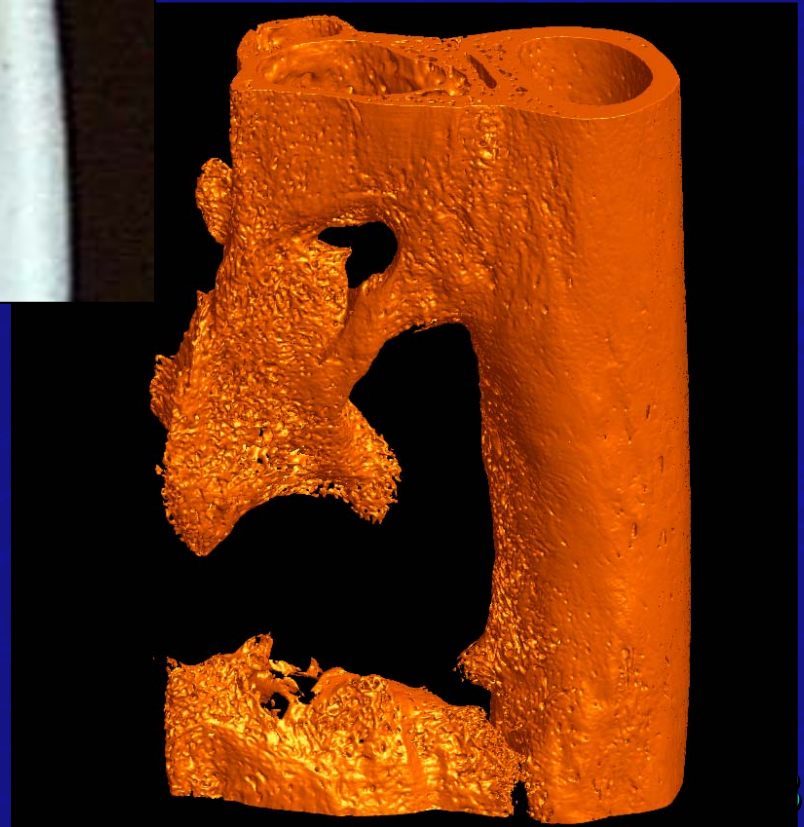


M. Sheller, et. Al., J Orthop Res **22**: 1094-9 (2005)

*Best of the
control
defects*



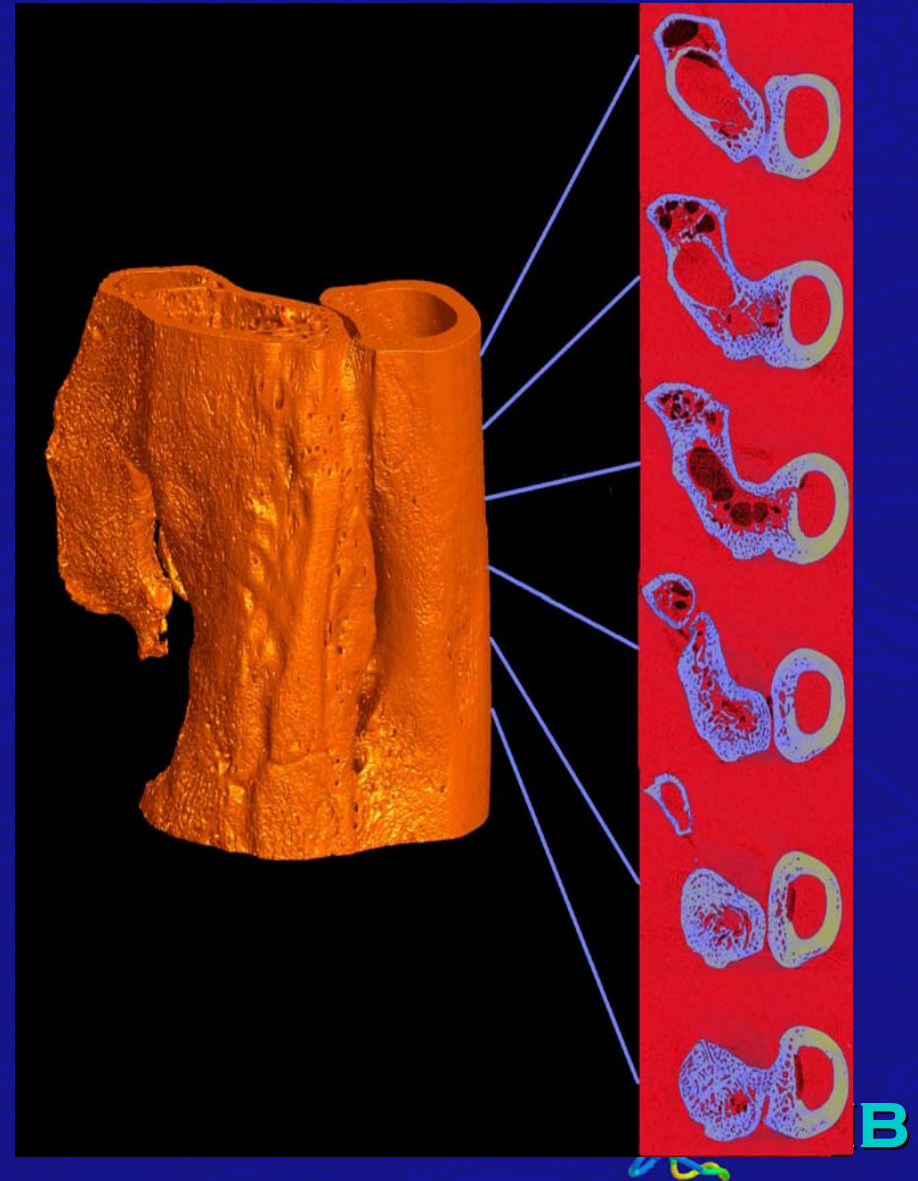
1010 - Control



TP508 stimulates bone regeneration



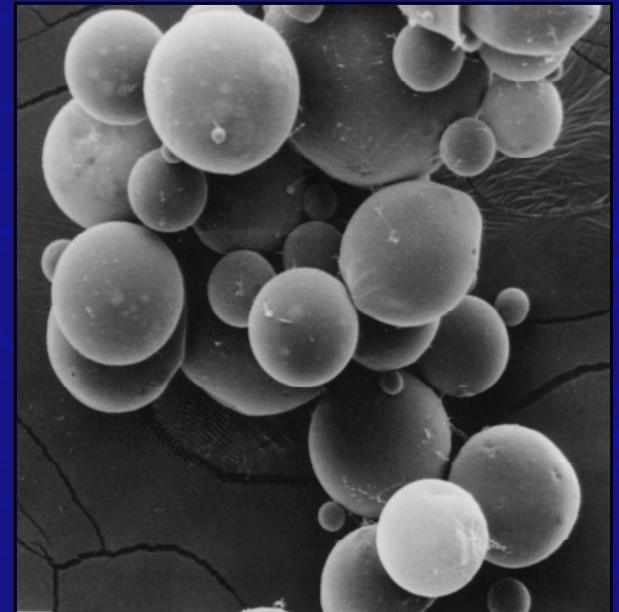
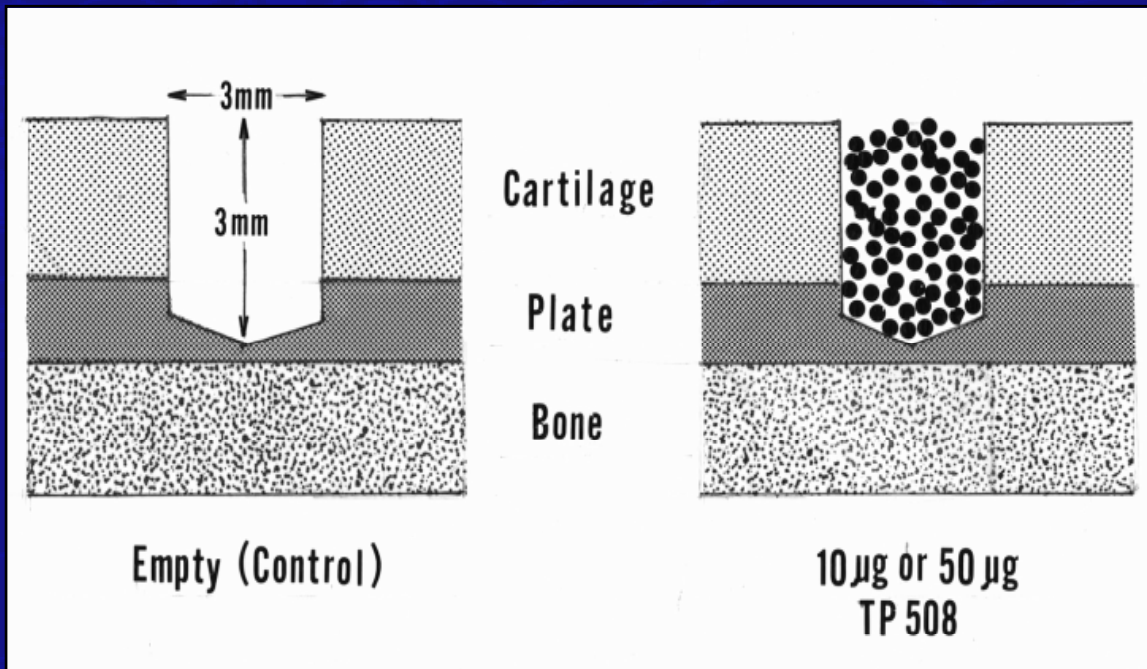
Chrysalin 100ug -
1009



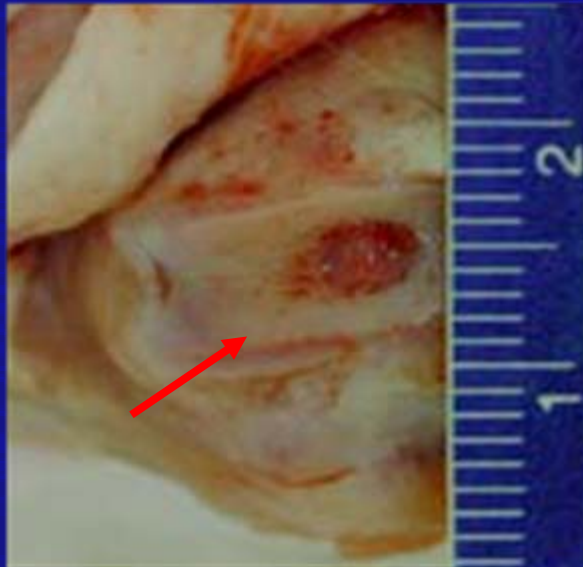
Preclinical Articular Cartilage Repair

Bilateral 3mm full thickness critical size defects in NZW rabbits
Dan Grande, New York

**TP508
Microspheres**



Effect of TP508 on Rabbit Cartilage Repair



Control



50µg TP508

- PLGA microspheres alone or with TP508 were implanted into 3mm defects in pluronic gel. Pictures show external appearance of trochlear groove surface after 4 weeks.



Orthopedic Clinical Trials

- **Fracture Repair**
- **Spine Fusion**

Distal Radius Fracture Trials Conducted by OrthoLogic

- Prospective, Double-blind, Randomized, Placebo- Controlled Trials
- Phase 1/2
 - 90 Patients in 7 centers
 - 3 Treatment Groups:
 - Chrysalin: 10 μ g (1 cc)
 - Chrysalin: 100 μ g (1 cc)
 - Placebo: saline (1 cc)
 - Single injection at time of fracture reduction
- Phase 3
 - 502 Patients
 - 2 groups Placebo and 10 μ g
 - Single injection at time of fracture reduction



Phase 1/2 Study

- No drug related adverse events
- Chrysalin treated subjects healed significantly (~30%) faster than controls

Phase 3 Study

- Radiographic evidence of healing (primary clinical endpoint not met)
- Clinical endpoints plus all radiographic endpoints showed significant effect of TP508 in osteopenic subpopulation of “at risk” subjects
- No drug related adverse events

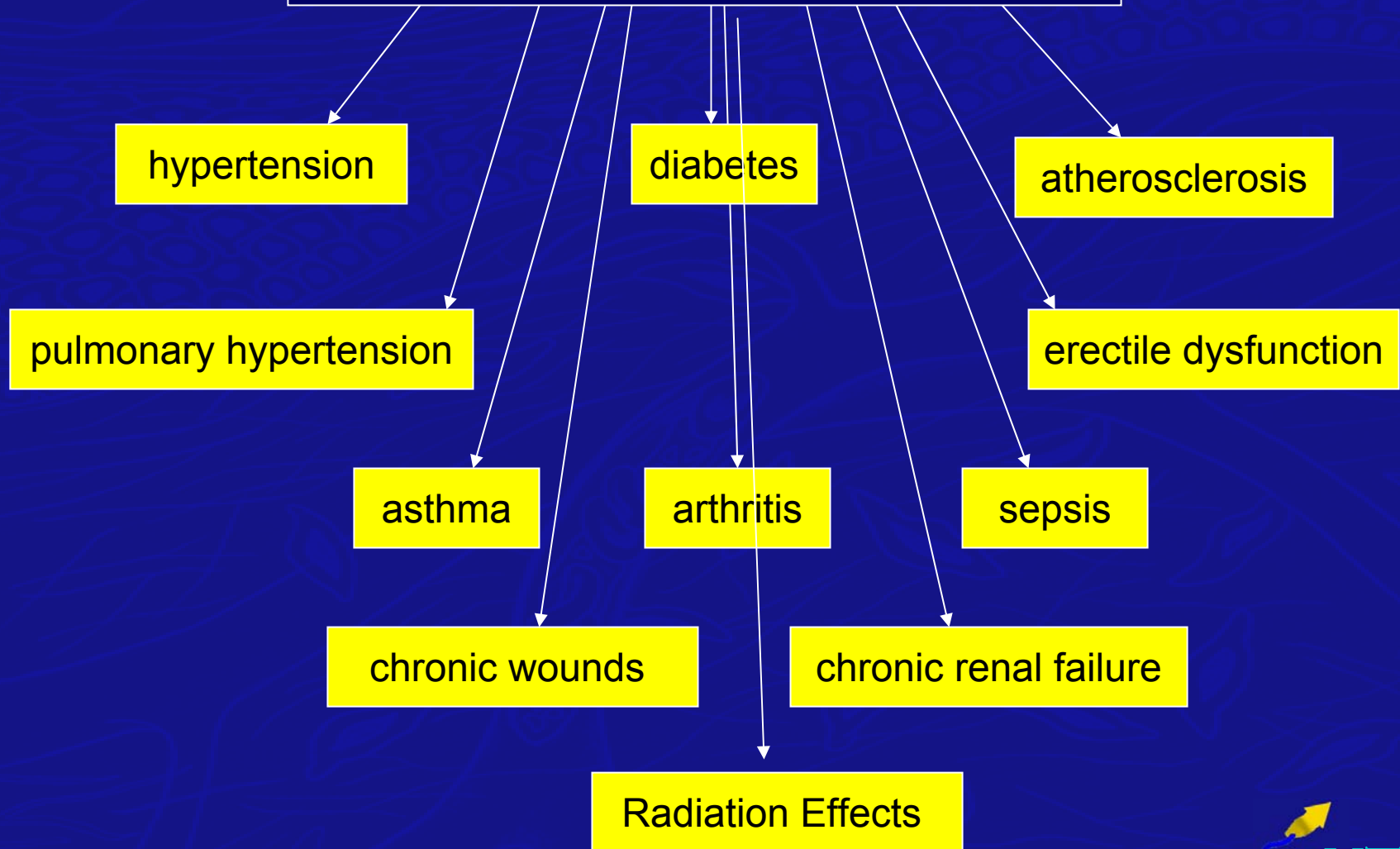
Is there a unifying mode of action that can explain the diverse effects of TP508?

- Endothelial effects appear to be seen in all tissues
- TP508 induces angiogenesis by itself or by enlisting other factors
- Diabetic foot ulcers fail to heal due to endothelial dysfunction, yet TP508 very effective in these chronic wounds
- Hypothesis that TP508 may effect NO signaling pathway and reverse endothelial dysfunction as part of its mode of action

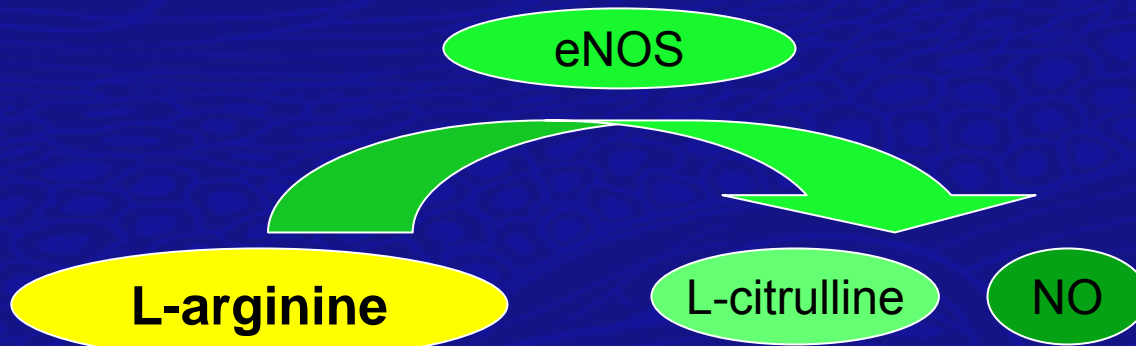
Endothelial Dysfunction (ED)

- Characterized by impaired NO production in EC
 - prevents vasodilation in response to agonists or blood flow
 - loss of response to angiogenic factors
 - loss of natural endothelial protection
- ED occurs in and contributes to many diseases including:
 - Hypertension
 - Diabetes
 - Chronic wounds
 - Cardiovascular disease
 - Atherogenesis
 - Pulmonary airway diseases
 - Asthma
 - Kidney failure
 - Erectile dysfunction
 - Cerebrovascular disorders
- ED involved in local and systemic radiation effects

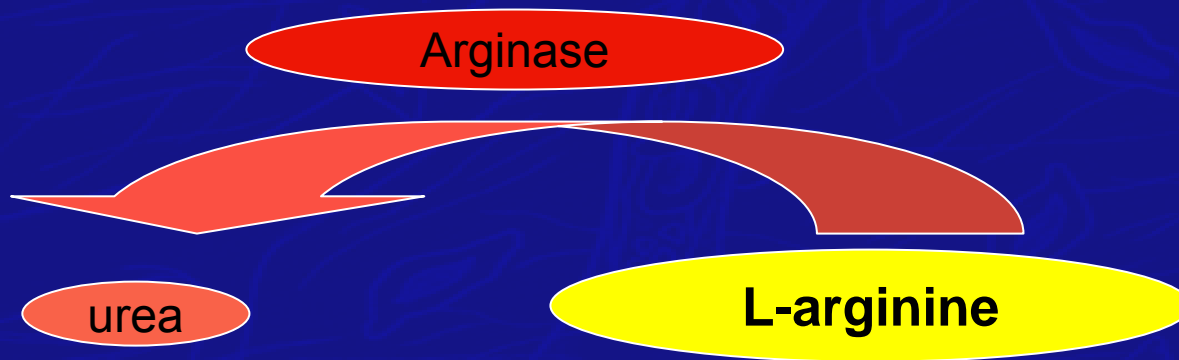
ENDOTHELIAL DYSFUNCTION



Nitric Oxide (NO) production in endothelial cells is regulated by multiple processes



- NO is produced from arginine by nitric oxide synthase (eNOS)
- In ED, eNOS activity is decreased by hypoxia and/or inflammation (TNF α)

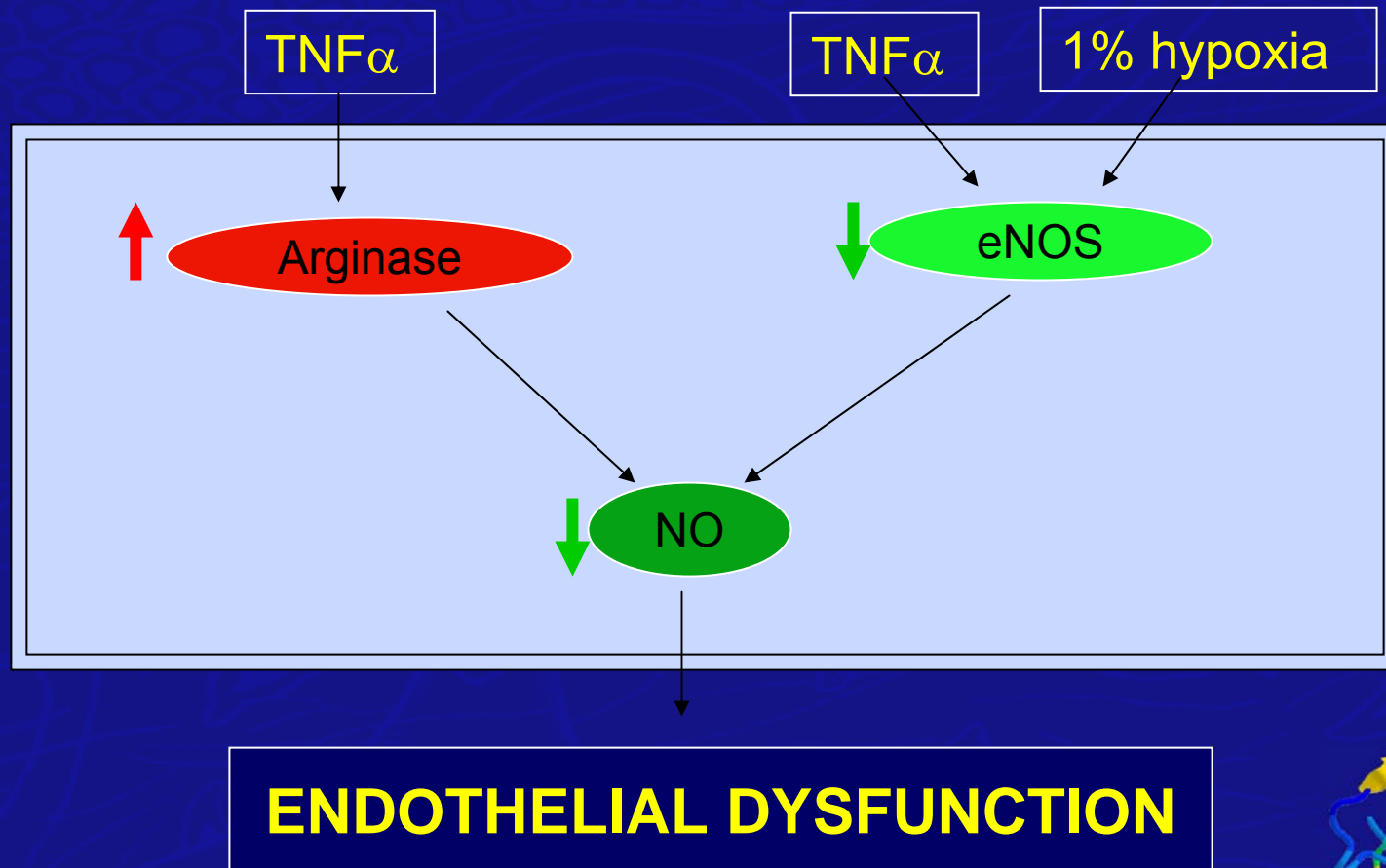


- arginase competes with eNOS for L-arginine
- In ED, arginase is increased depleting arginine and reducing NO

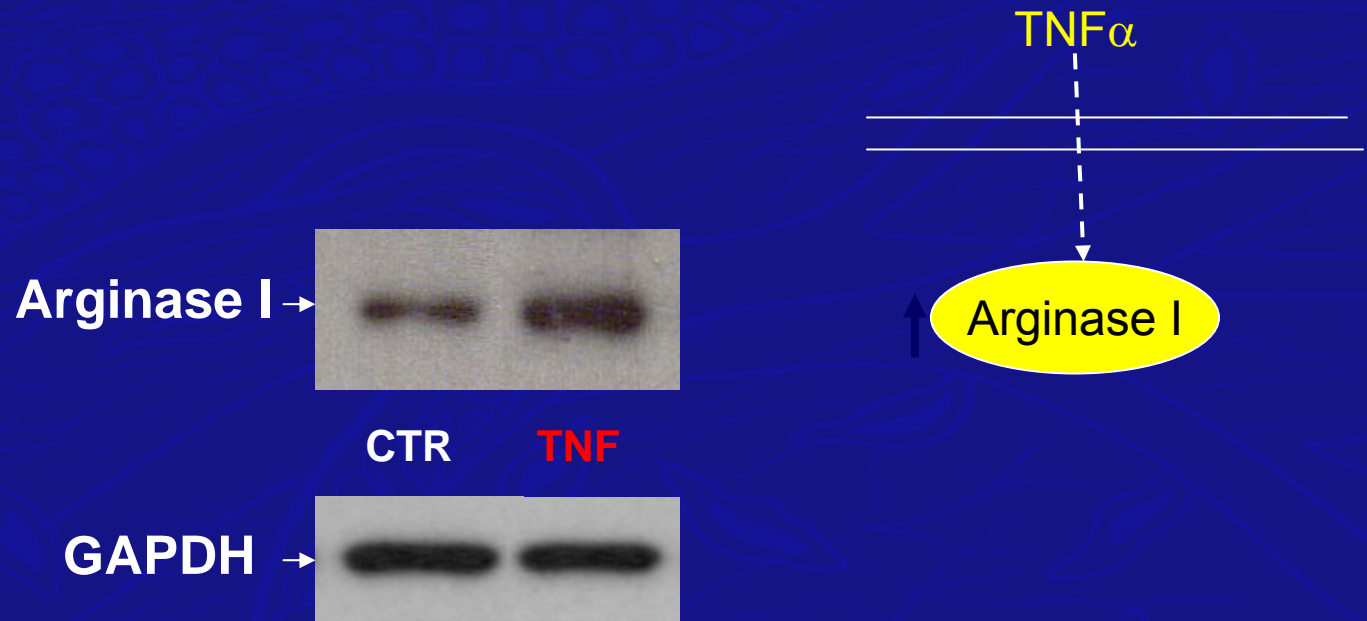
In vitro model of endothelial dysfunction

Human Aortic Endothelial Cells

Human Coronary Artery Endothelial Cells

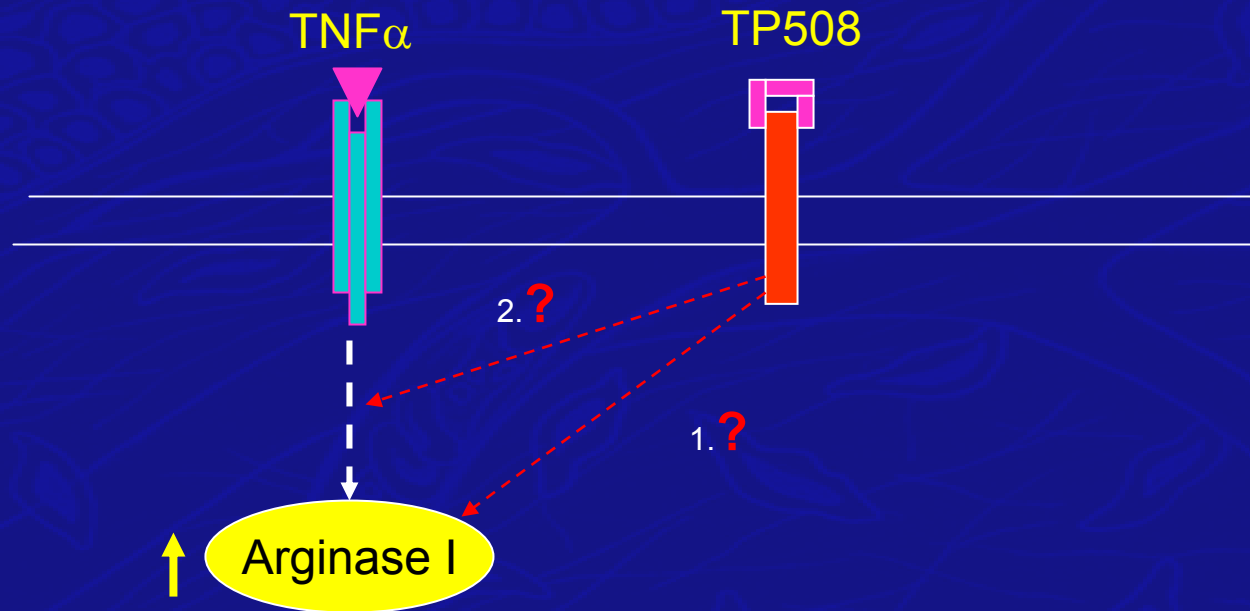


TNF up-regulates of Arginase I expression in Human Coronary Artery Endothelial Cells

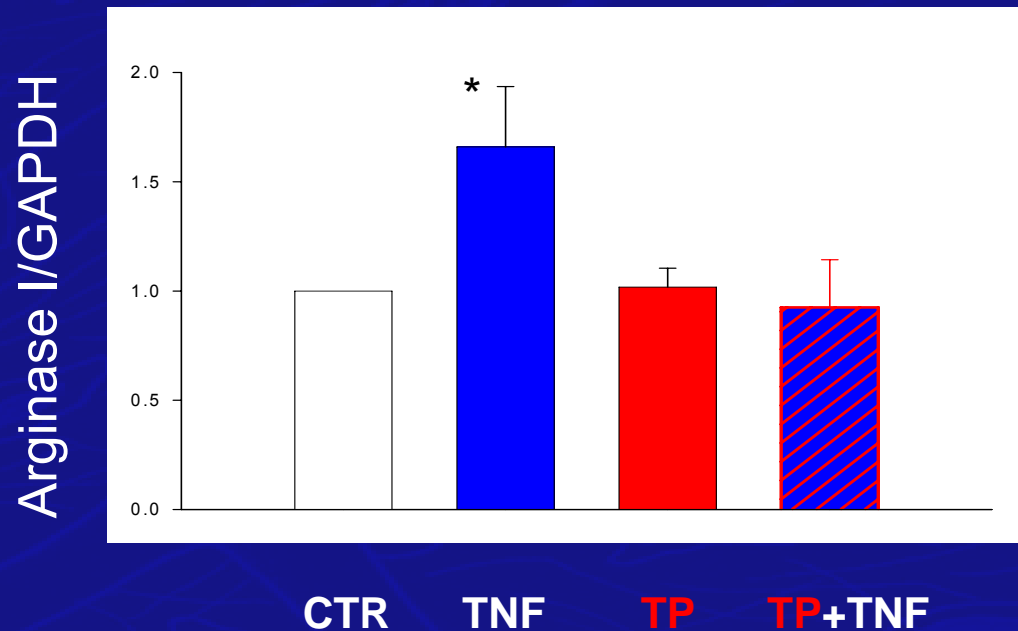
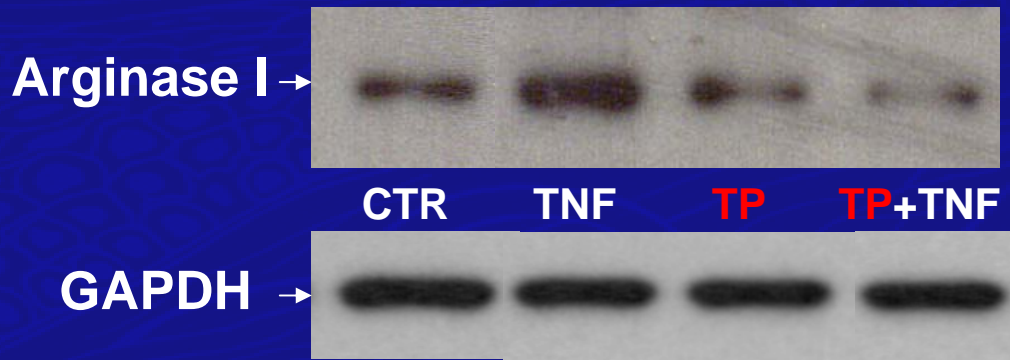


Barbara Olszewska-Pazdrak, MD

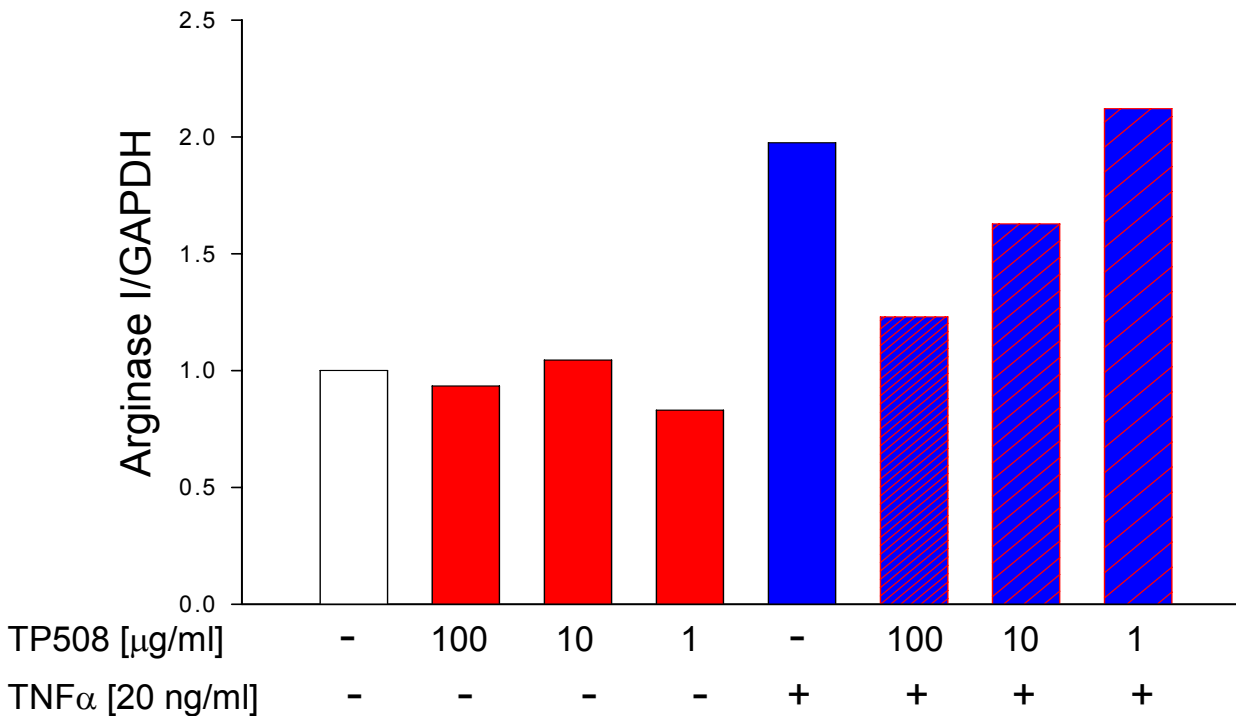
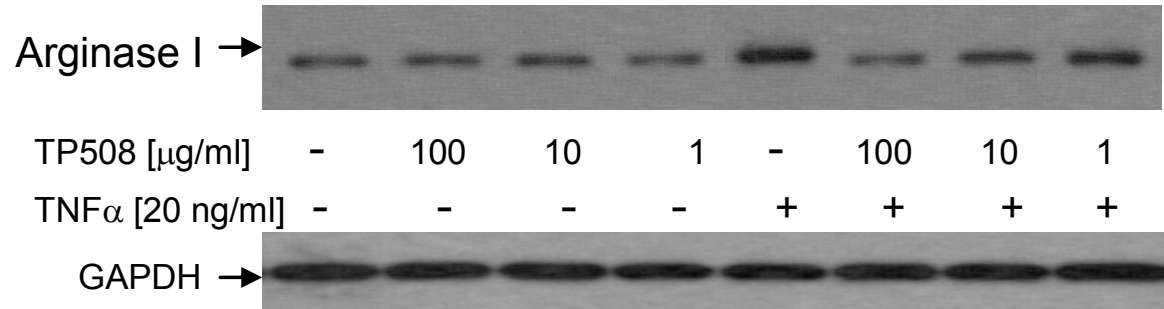
Does TP508 alter TNF α -induced up-regulation of Arginase I expression?



TP508 prevents TNF-induced up-regulation of Arginase I expression in Human Coronary Artery Endothelial Cells



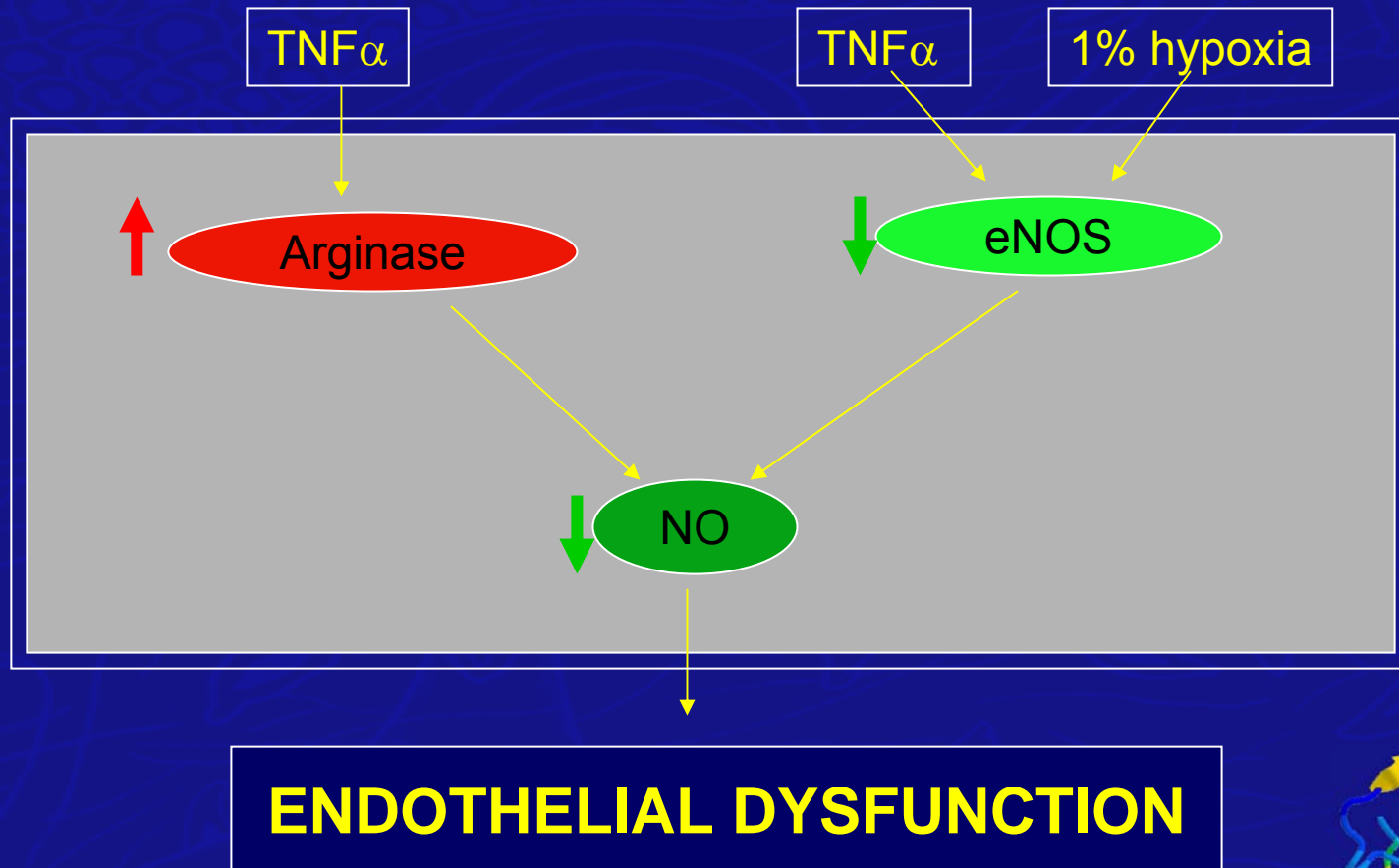
Dose-dependent inhibition of $TNF\alpha$ -induced up-regulation of arginase I by TP508



In vitro model of endothelial dysfunction

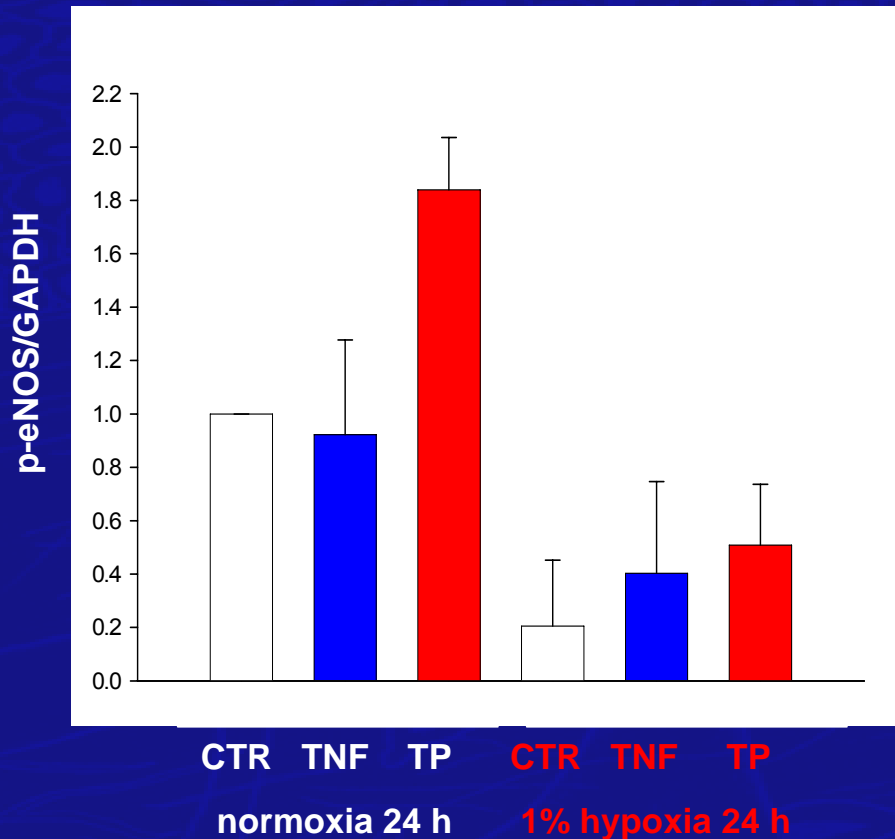
Human Aortic Endothelial Cells

Human Coronary Artery Endothelial Cells

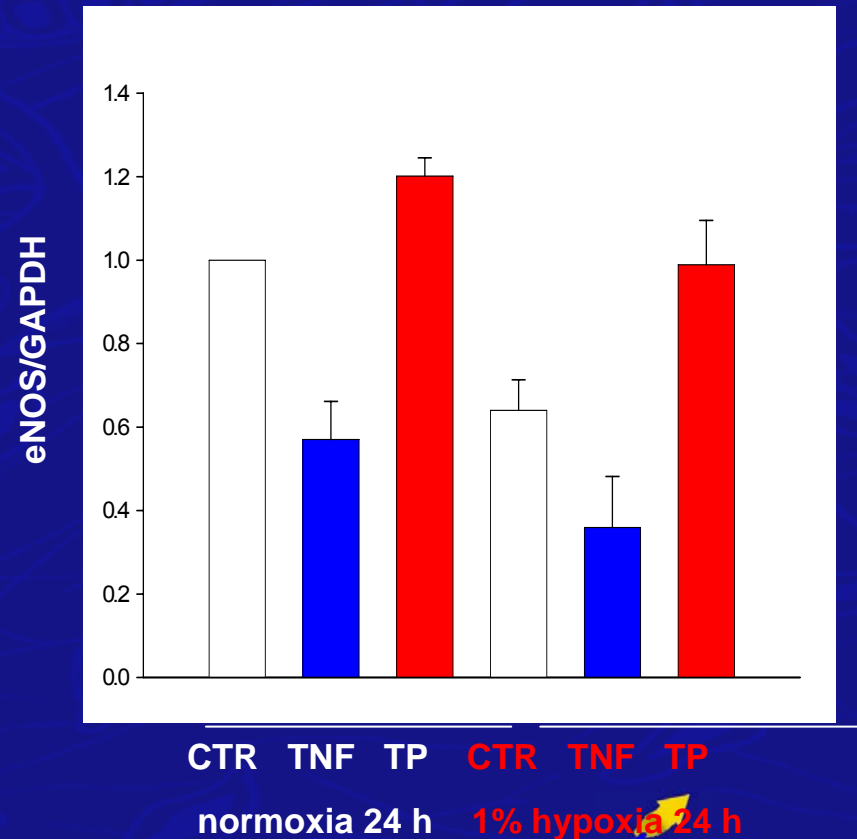


Effect of TP508 on eNOS activation and expression in human endothelial cells cultured in normoxic or hypoxic conditions

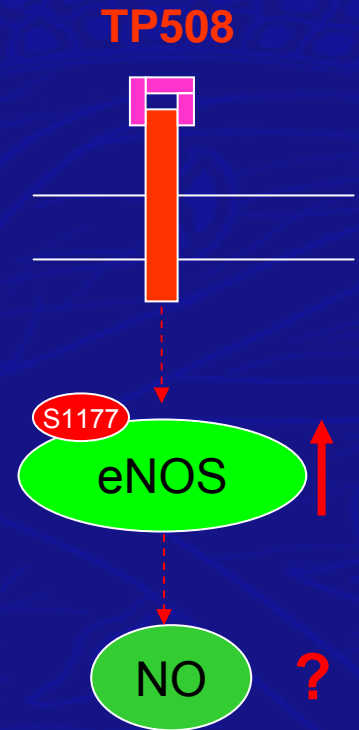
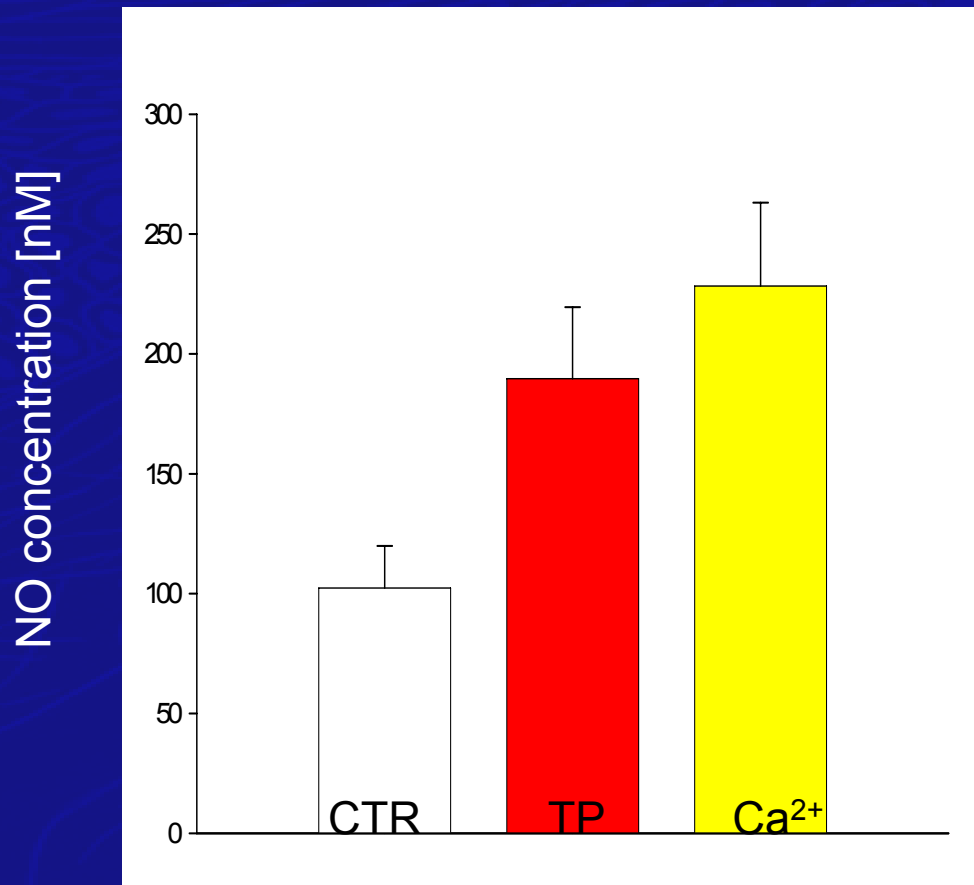
eNOS phosphorylation



eNOS protein expression



TP508 increases NO production by human endothelial cells



TP508 Effects on eNOS and Arginase

■ Arginase

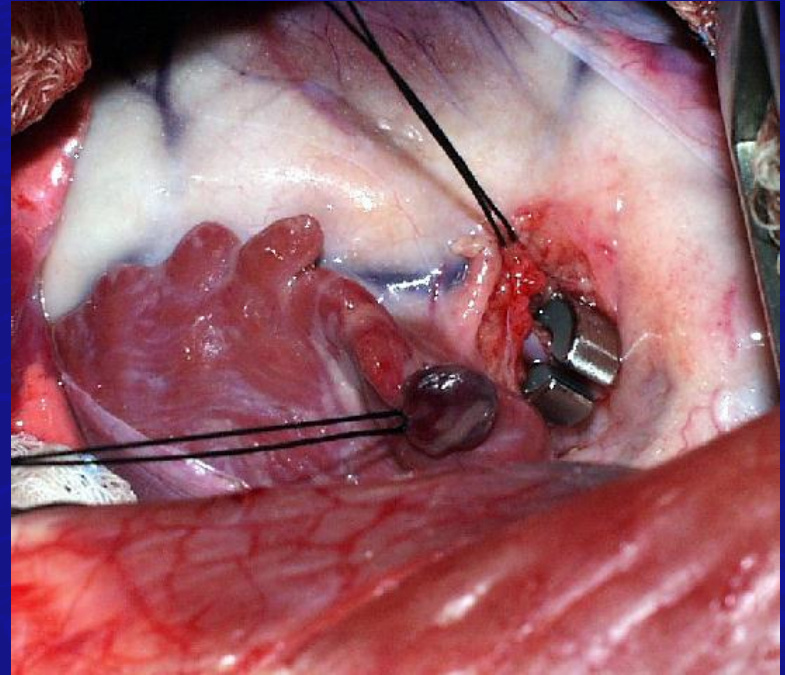
- TP508 blocks the upregulation of arginase induced by $\text{TNF}\alpha$
- Effect is dose dependent

■ eNOS

- In normoxia TP508 stimulates phosphorylation of eNOS
- In hypoxic cells TP508 upregulates (or prevents down-regulation) of eNOS increasing the enzyme levels
- Effects of TP508 on both phosphorylation and enzyme levels are dose dependent
- TP508 increases phosphorylation of eNOS even in the presence of $\text{TNF}\alpha$

Ischemic Heart Model -- Functional Evaluation

**Yucatan crossbreed
mini-pigs**

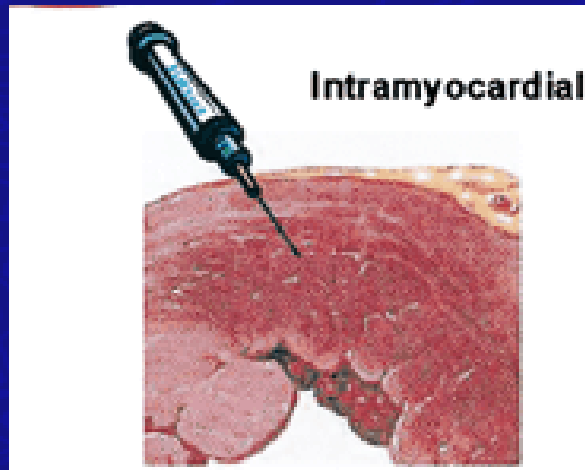


**Ameroid
constriction of
proximal LCX**

**Theresa Fossum, DVM, PhD, Texas A&M
University, College of Veterinary Medicine**



Endothelial dysfunction and myocardial revascularization



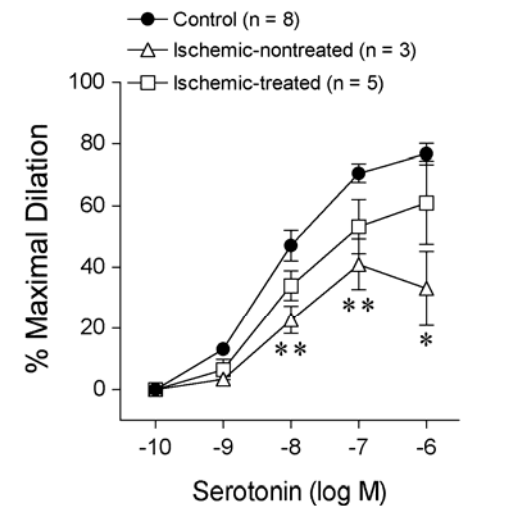
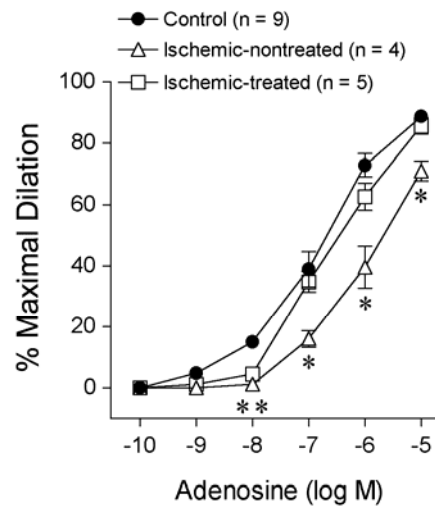
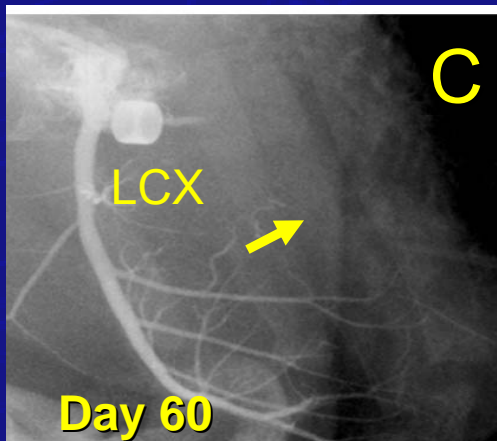
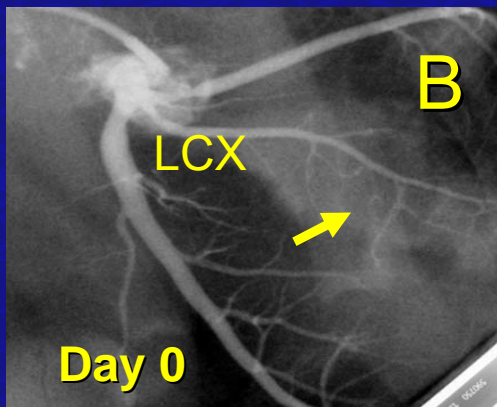
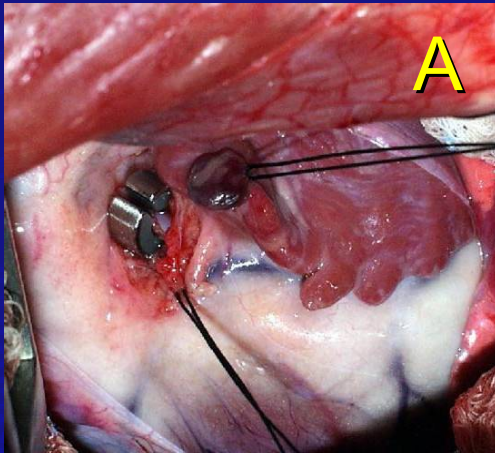
At 30 days

- Myocardial injection of Chrysalin using controlled release formulation

At 60 days

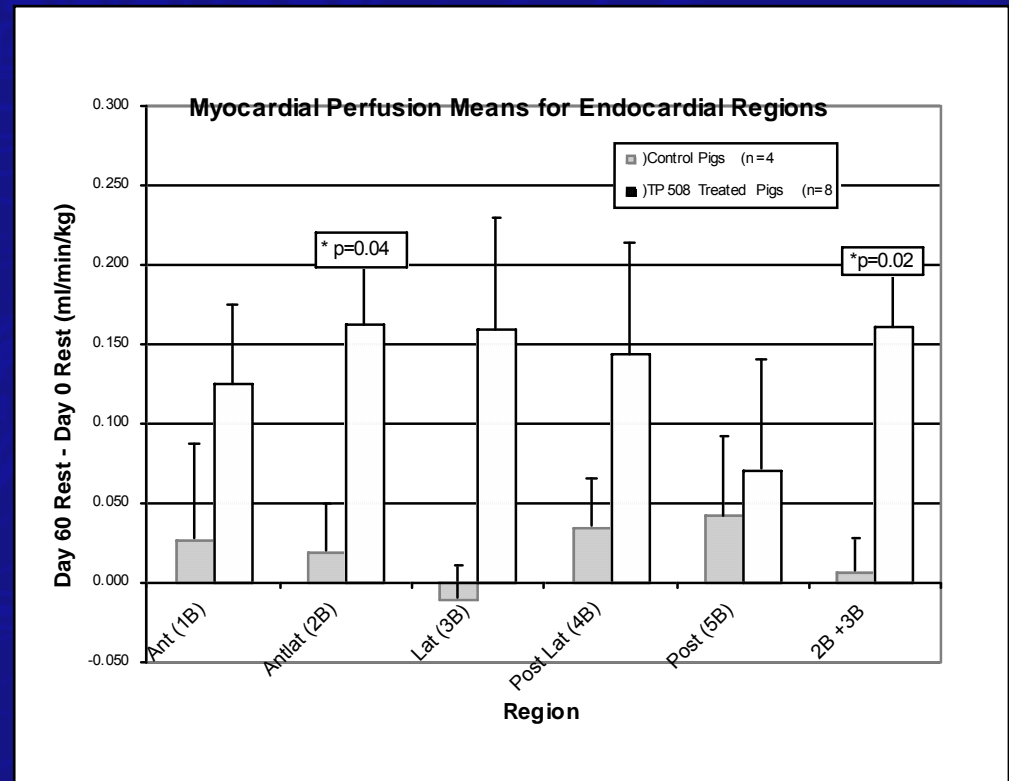
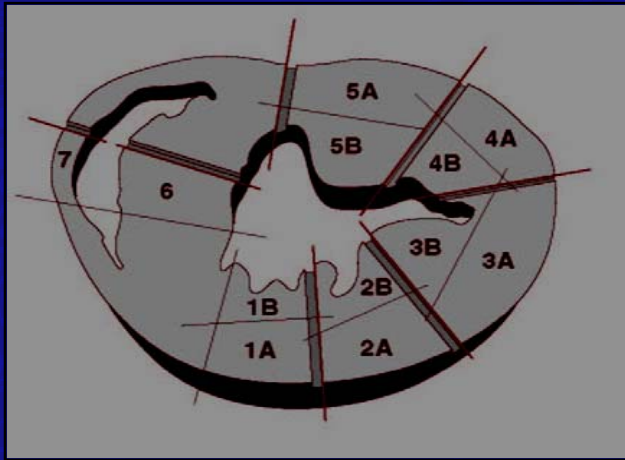
- Isolate arterioles and test for endothelial dysfunction
- Perfusion data
- Cardiac function
 - Echocardiography

TP508 reverses ED in Coronary Arterioles

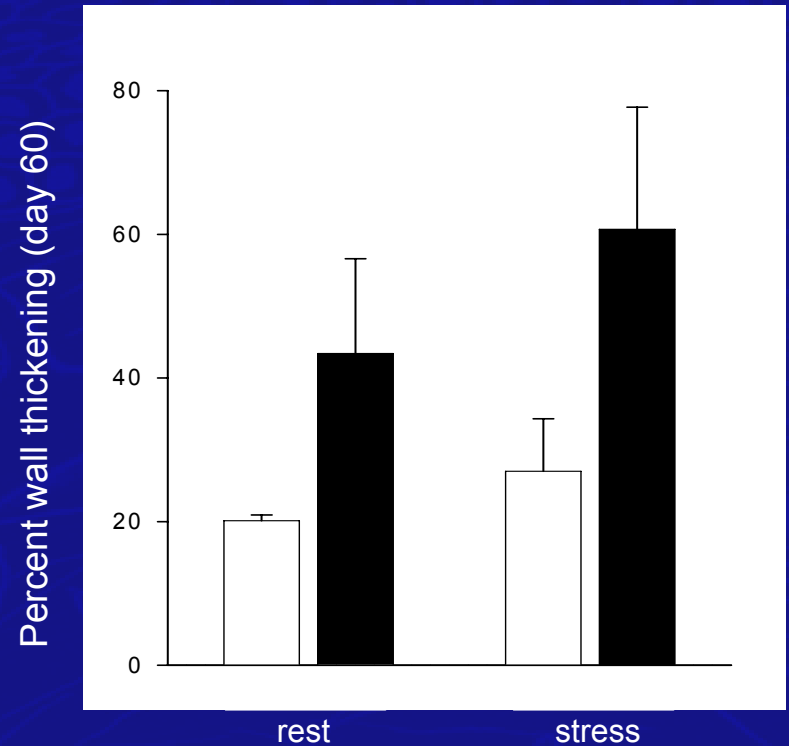
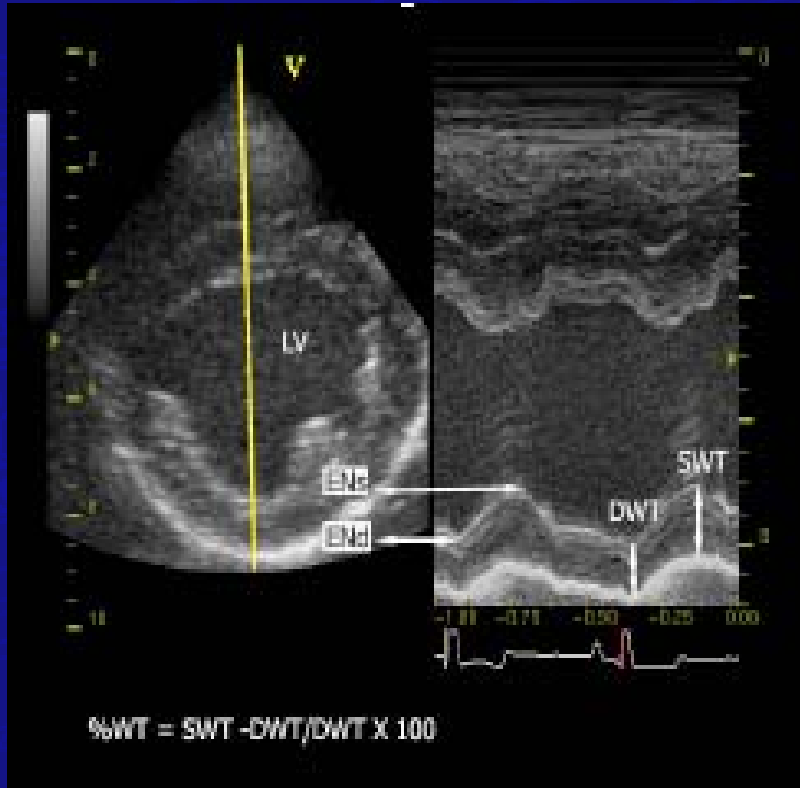


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TP508 increases perfusion and Revascularization



TP508 Promotes Functional Recovery



Echocardiograms at 60 days measure % change in wall thickening which correlates with ability of muscle to contract

Myocardial Ischemia is first in vivo demonstration of ED reversal

- Functional response (perfusion & wall thickening) of ischemic myocardium significantly increased
- Chrysalin restores NO signaling pathway responsiveness in vessels isolated from ischemic heart
- Results may have implications for dermal and orthopedic applications in aging populations and in radiation combination injuries where endothelial dysfunction may affect revascularization and healing